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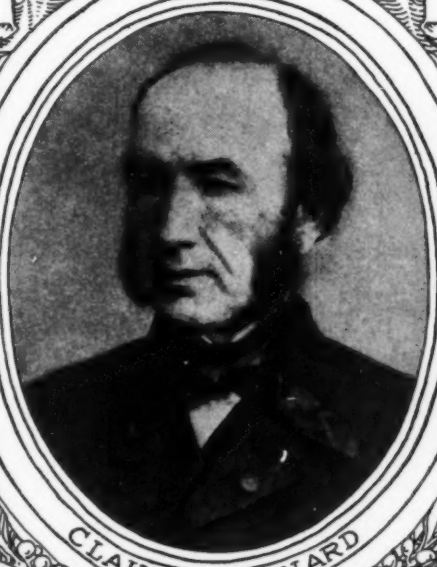

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THE UTILIZATION BY CALVES OF THE ENERGY CONTAINED IN BALANCED RATIONS COMPOSED OF COMBINATIONS OF DIFFERENT FEEDS

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(Received for publication July 14, 1941)

The investigation to be reported in this paper was undertaken to test with growing calves the proposition that, although the energy wastages of rations in digestion, aside from the associative effects of one feed upon the digestibility of another, are characteristics of the various feeds of which they are composed, as expressed in their chemical compositions, the energy wastages of rations in metabolism are not characteristic functions of the constituent feeds but rather are functions of the combinations of digestible nutrients supplied to the tissues of the body. To the extent that these combinations satisfy the nutritive requirements of the tissues, the metabolizable energy of the digestible nutrients will be the more perfectly utilized and less will be wasted. If the mixture of digestible nutrients is adequate for the maximum performance of all tissue functions, then the utilization of metabolizable energy will be maximal. If it is inadequate in respect to one or more essential nutrients, eventually the wastage of metabolizable energy will increase, and conceivably the metabolizable energy may be completely wasted as animal heat (specific dynamic effect). This eventuality may be deferred until the body's stores of the lacking nutrients become exhausted; and in some animals bred through generations to fatten readily, a further deferment may result from the ready transformation

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of organic nutrients into body fat, irrespective of their balanced character (Mitchell, Hamilton and Haines, '40).

The rational basis for this proposition (Mitchell, '34) and its historical development and evidential support (Mitchell, '37) have been presented by the senior author, while the junior author has reported the results of an investigation illustrating clearly its validity in rodent nutrition (Hamilton, '39).

If the above proposition is correct, animal feeds (and human foods also) do not possess distinctive net energy values, separately determinable and additive in estimating the net energy contained in rations or diets. Their contents of metabolizable energy are more or less characteristic, but the net availability of the metabolizable energy depends upon the nutritive adequacy of the metabolizable nutrients provided by the rations of which the feeds are components. Hence, the metabolizable energy in rations equally well-balanced with reference to the nutritive requirements of the test animal, should be equally well utilized, regardless of the number or kinds of feeds combined in the rations. This particular aspect of the problem was the object of the experiments described below.

PLAN OF THE EXPERIMENTS

Four rations were designed (1) to contain similar proportions of the various classes of nutrients as determined by proximate chemical analysis, (2) to contain combinations of different feeds, no one feed occurring in more than one ration, and (3) to be adequate in all essential nutrients.

The main constituents of the rations and their proximate chemical composition are given in table 1. All rations were supplemented with 56 gm. of steamed bone meal and 10 gm. of a fortified cod liver oil [1] per head daily, while rations A and B were supplemented in addition with 100 gm. per head daily of a dried yeast.[2]

[1] Nopco XX.
[2] Northwestern.

The utilization of the gross energy in each ration was determined upon four grade Shorthorn calves. Rations A and B were tested in 1938–1939 on one group of four calves, and rations C and D in the following year on another group of four calves.

The tests involved either two or three determinations of the fasting heat production of the calves, carried out on the fourth and fifth days of fast if the values for the 2 days agreed

TABLE 1

Proximate chemical composition of the experimental rations.

RATION	DRY MATTER	CRUDE PROTEIN	ETHER EXTRACT [1]	ASH [1]	CRUDE FIBER [1]	NITROGEN FREE EXTRACT [1]	GROSS ENERGY
	%	%	%	%	%	%	*cal./gm.*
Ration A: alfalfa hay 30, corn 40, linseed meal 30	90.54	18.71	4.24	4.41	9.30	53.87	4.156
Ration B: timothy hay 30, barley 50, meat scraps 20	91.67	19.87	2.98	9.12	12.91	47.90	4.089
Ration C: red clover hay 15, oats 60, cottonseed meal 25	91.51	18.61	5.01	4.72	13.08	49.36	4.141
Ration D: oat straw 20, wheat 45, soybean oil meal 20, whole pressed cottonseed 15	91.47	20.83	3.28	3.86	13.18	50.80	4.086

[1] Computed from average analyses taken from Morrison's "Feeds and Feeding."

within 2%, or on the fourth, fifth and sixth days in the unusual event that such agreement was not attained. The averages thus secured, expressed to the $W_{kg.}^{.75}$, served as bases for the estimation of the heat increments of feeding for the various test rations. The validity of this procedure, in the opinion of the writers, has not been successfully challenged and, in fact, may be justified on practical grounds regardless of the existence or non-existence of a functional minimum level of metabolism less than the fasting metabolism by a quantity of heat originating from the specific dynamic action of the body nutrients undergoing metabolism (Forbes and Swift, '41).

The utilization of energy in the four test rations was carried out in a metabolism period extending over 12 to 14 days (preceded by a preliminary period of at least 7 days), in which the gross energy in feed, feces, and urine was determined, and in a 2- or 3-day period in the respiration chamber, in which the heat production was determined from the daily consumption of oxygen and the daily production of carbon dioxide and methane. The respiration experiment was terminated after 2 days if the values per 24 hours agreed within 2%. In most cases, the determination of heat production followed the digestion and metabolism test, and in those cases where this was not true, it was preceded by a preliminary feeding period of at least 7 days. Each ration was tested at two planes of nutrition, one estimated to approximate energy equilibrium and the other supermaintenance.

The schedule of the experiments, indicating merely the order in which the fasting heat production and the various ration tests were carried out, together with the average body weights of the steers while in the respiration chamber, is given in table 2. An attempt was made to avoid possible age effects by testing each ration in one order with two steers and in the reverse order with the other two steers. The high and the low planes of nutrition for each ration were also tested in different orders for the same reason.

The steers were confined in metabolism stalls maintained at a temperature of 75°F. and at a humidity of approximately 50% and were fed twice daily. They were weighed and watered each morning before feeding and watered again before the second feeding. The heat production was determined in an open-circuit respiration chamber previously described (Mitchell et al., '32).

All rations during each period, and composite samples of all collections of feces and urine, were analyzed for nitrogen and their heats of combustion (gross energy) measured in an adiabatic Parr oxygen-bomb calorimeter. The dry matter content of all rations was also determined.

All calorimetric calculations were made on the basis of daily heat productions corrected to a standard day of 12 hours standing and 12 hours lying down, following the procedure introduced by Armsby.

TABLE 2

Experimental schedule for the 2 years of work.

PERIOD	STEER 37-1		STEER 37-2		STEER 37-3		STEER 37-4	
	Ration and plane of nutrition [1]	Body weight	Ration and plane of nutrition [1]	Body weight	Ration and plane of nutrition [1]	Body weight	Ration and plane of nutrition [1]	Body weight
		kg.		*kg.*		*kg.*		*kg.*
1	A—H	316	A—L	271	B—H	319	B—L	307
2	Fast	300	Fast	268	Fast	306	Fast	290
3	A—L	306	A—H	326	B—L	295	B—H	359
4	Fast	295	B—H	341	A—L	304	A—H	383
5	B—L	303	Fast	333	Fast	294	Fast	382
6	B—H	347	B—L	329	A—H	334	A—L	368
7	Fast	331	Fast	318	Fast	325	Fast	369
	STEER 40-1		STEER 40-2		STEER 40-3		STEER 40-4	
1	Fast	238	Fast	231	Fast	254	Fast	219
2	C—L	241	C—L	235	D—L	265	D—L	228
3	C—H	277	C—H	269	D—H	296	D—H	256
4	D—H	303	D—H	295	C—H	312	C—H	267
5	D—L	294	D—L	275	C—L	304	C—L	260
6	Fast	283	Fast	274	Fast	306	Fast	260

[1] L = low plane; H = higher plane.

EXPERIMENTAL RESULTS

The average contents of metabolizable energy per kilogram of dry matter and the average apparent digestibility of nitrogen for the four experimental rations at the two levels of feeding are presented in table 3. In respect to these items, the rations did not differ greatly, but for reasons that are not at all evident, the methane production on rations C and D, expressed as the gross energy of the methane produced per kilogram of dry matter in the ration, was much greater than that on rations A and B, indicating a more active paunch fermentation for the former rations. It should also be noted that rations A and B were more readily consumed than rations C and D, and in particular that higher levels could be fed because of their greater palatability. Also, ration A proved to be more palatable than ration B.

The fasting heat production of the steers, expressed in calories per $W_{kg.}^{.75}$, is given in table 4. The average value for each steer has been used in computing heat increments of feed for the experimental rations tested. The average values for all

TABLE 3

Metabolizability of energy and digestibility of nitrogen of the experimental rations. Averages for four steers.

RATION	GROSS ENERGY CONSUMED	METABOLIZABLE ENERGY		METHANE ENERGY PER KILOGRAM OF DRY MATTER	APPARENT DIGESTIBILITY OF NITROGEN
		Per kilogram of dry matter	Percentage of gross energy		
	cal.	*cal.*	%	*cal.*	%
		At lower plane of nutrition			
A	14184	2886	63.54	400	76.63
B	12040	2650	59.92	422	81.06
C	11382	2408	52.92	722	74.26
D	11499	2471	55.25	763	79.32
		At higher plane of nutrition			
A	27654	2911	63.97	303	72.64
B	23206	2785	62.63	370	78.82
C	21568	2419	54.17	606	72.55
D	20873	2512	56.86	644	77.12

TABLE 4

The fasting heat production of the steers, expressed in calories per $W_{kg.}^{.75}$

STEER NO.	TEST	FASTING HEAT PRODUCTION	STEER NO.	TEST	FASTING HEAT PRODUCTION
		cal.			*cal.*
37-1	1	86.93	40-1	1	94.82
	2	79.36		2	90.90
	3	77.66		Average	92.86
	Average	81.32			
37-2	1	69.17	40-2	1	77.74
	2	78.97		2	74.16
	3	71.70		Average	75.95
	Average	73.28			
37-3	1	72.16			
	2	69.57	40-3	1	82.01
	3	77.22		2	75.02
	Average	72.98		Average	78.52
37-4	1	69.08			
	2	76.36	40-4	1	86.75
	3	70.13		2	75.88
	Average	71.86		Average	81.32

steers was 78.51 cal. per kilogram of $W^{.75}$. This value is higher than the average recently obtained (Mitchell, Hamilton and Haines, '40) for four somewhat lighter steer calves, i.e., 71.75 cal.

The most significant energy metabolism data secured in the respiration chamber with reference to the four experimental rations and the two planes of nutrition are summarized in table 5. The percentage net availability of the metabolizable energy was quite similar for the four experimental rations at the lower plane of nutrition and also at the higher plane of nutrition. In accordance with previous findings with reference to the effect of the plane of nutrition on the utilization of food energy (Forbes et al., '28, '30; Wiegner and Ghoneim, '30; Mitchell et al., '32), the net availability of metabolizable energy at the higher plane of nutrition was lower than that at the lower plane.[3]

As the values in the last column of table 5 reveal, the plane of nutrition varied considerably among the experiments in which the different rations were tested, even within the two series of experiments at so-called "low" and "high" planes of nutrition. A more exact comparison of the utilization of metabolizable energy in the four rations would be afforded if computations could be made for the point of energy equilibrium, and for levels above this point, computing heat increments from the heat production at an exact maintenance level of feeding.

This may be done by fitting the following equation to the eight sets of data secured for each experimental ration:

$$m = ax + by \qquad (1)$$

in which m is the total intake of metabolizable energy, a is the estimated basal heat production at the prevailing body weight, b is the energy balance if positive, and x and y are the amounts of metabolizable energy expressed in calories presumably

[3] The average percentage difference in heat production between successive days in the calorimeter, for the feeding tests as well as the fasting tests, was 1.49, and in 73% of the tests it was less than 2.

TABLE 5

The utilization of metabolizable energy of the experimental rations.

RATION	STEER	AT THE LOWER PLANE OF NUTRITION						AT THE HIGHER PLANE OF NUTRITION					
		Estimated basal heat production	Metabolizable energy consumed	Heat increment [1]	Net energy		Plane of nutrition [2]	Estimated basal heat production	Metabolizable energy consumed	Heat increment [1]	Net energy		Plane of nutrition [2]
					Total	Fraction of metabolizable energy					Total	Fraction of metabolizable energy	
		cal.	*cal.*	*cal.*	*cal.*	%	%	*cal.*	*cal.*	*cal.*	*cal.*	%	%
A	37-1	5953	9406	2140	7266	77.2	122	6093	20995	6850	14145	67.4	232
	37-2	4893	9171	3027	6144	67.0	126	5617	16023	6204	9819	61.3	175
	37-3	5313	7439	2003	5436	73.1	102	5695	16130	5844	10286	63.8	181
	37-4	6040	9997	2932	7065	70.7	117	6224	17790	5875	11915	67.0	191
	Average					72.0						64.9	
B	37-1	5898	7182	1223	5959	83.0	101	6531	15979	5740	10239	64.1	157
	37-2	5654	7286	1954	5332	73.2	94	5816	14061	5144	8917	63.4	153
	37-3	5199	6904	2219	4685	67.9	90	5509	13074	4317	8757	67.0	159
	37-4	5272	7234	2496	4738	65.5	90	5924	15037	5268	9769	65.0	165
	Average					72.4						64.9	
C	40-1	5677	5775	1645	4130	71.5	73	6307	11481	3935	7546	65.7	120
	40-2	4551	5177	1647	3530	68.2	78	5048	11231	4244	6987	62.2	138
	40-3	5714	7064	2064	5000	70.8	87	5833	13217	5329	7888	59.7	135
	40-4	5267	6108	1458	4650	76.1	88	5365	10842	4485	6357	58.6	119
	Average					71.7						61.6	
D	40-1	6585	7204	1673	5531	76.8	84	6745	12642	3897	8745	69.2	130
	40-2	5133	7097	2460	4637	65.3	90	5269	12273	5057	7216	58.8	137
	40-3	5156	5919	2281	3638	61.5	71	5606	11821	5064	6757	57.2	121
	40-4	4774	5242	1647	3595	68.6	75	5209	10728	4927	5801	54.1	111
	Average					68.0						59.8	

[1] The difference between the observed heat production and the estimated basal heat production at the prevailing body weight.

[2] The net energy intake expressed as a percentage of the estimated basal heat production at the prevailing body weight.

required per calorie of basal heat (maintenance) and per calorie of energy balance (body increase), respectively. This equation expresses a purely conventional picture of the energy metabolism, a picture possessing certain pragmatic advantages in animal nutrition but questionable rational or evidential support (Mitchell, '27). Its pragmatic value depends upon the fact that the relationship between the heat production of an animal and its food consumption is curvilinear at low levels of intake, but at or near the point of energy equilibrium it is rectilinear, or approximately so, for a considerable range of food intake above maintenance (Brody and Procter, '33).

In the above equation, the value of x can be assumed to be constant only for planes of nutrition above maintenance. When the energy balance is negative, x would tend to decrease, because of the better utilization of feed energy in sub-maintenance nutrition. Hence, before fitting the above equation to the four sets of data for the four experimental rations, a correction for this fact had to be made in those cases in which the energy balance was negative.

First, the general nature of the relationship between the utilization of metabolizable energy, $\frac{Q_n}{Q_m} \times 100$, and the plane of the nutrition, Q_p, as measured by the net energy intake expressed as a percentage of the basal heat production, was determined by fitting to all of the data for all of the rations an equation of the type found to be suitable for this purpose by Brody and Procter ('33). Using the method of least squares in this procedure, the following equation resulted:

$$\frac{Q_n}{Q_m} \times 100 = 77.00\, e^{-0.001166\, Q_p} \qquad (2)$$

According to this equation, at energy equilibrium, $Q_p = 100$, the net availability of metabolizable energy, $\frac{Q_n}{Q_m} \times 100$, equals 68.32. In correcting the value of m (equation 1) for any set of data for which the plane of nutrition is less than 100, the plane of nutrition, Q_p, is substituted in equation 2 and the equation is solved for $\frac{Q_n}{Q_m} \times 100$. Then the observed percentage utilization of the metabolizable energy (table 5) is lowered

by an amount equal to the difference between 68.32 and the value of $\frac{Q_n}{Q_m} \times 100$ thus found. Applying this percentage to the basal heat production of the steer gives the corrected m value.

To the observed data of metabolizable energy, basal heat production and energy balance, corrected as explained above where correction was needed, for each experimental ration, equation 1 was fitted by the method of least squares, with the results shown in the second and third columns of table 6. The

TABLE 6

Estimated net availability of metabolizable energy for maintenance and for body increase.

RATION	NET AVAILABILITY OF METABOLIZABLE ENERGY			
	Unadjusted estimates		Estimates adjusted to constant rate of paunch fermentation [1]	
	Maintenance	Body increase	Maintenance	Body increase
	%	%	%	%
A	72.5	60.3	72.6	59.5
B	72.3	55.1	72.8	54.4
C	69.6	44.3	71.2	44.2
D	64.4	53.7	66.0	53.3
Average	69.68	53.34	70.67	52.84

[1] Four hundred calories produced as methane per kilogram of dry ration.

net availability of metabolizable energy for maintenance is equal to $100 \div x$, while that for body increase is equal to $100 \div y$.

It will be noted that the percentage utilizability of metabolizable energy, for both maintenance and body increase, is lower for rations C and D than for rations A and B. This might possibly be a result of the greater paunch fermentation per kilogram of dry matter consumed observed with rations C and D than with rations A and B (table 3). A greater production of methane is presumably associated with a greater heat of fermentation, which, while actually non-metabolizable, is of necessity included in the metabolizable energy. Hence, the metabolizable energy in all tests and all animals was adjusted to a constant methane production, equivalent to 400 methane calories per kilogram of dry matter consumed, on the basis of an assumed ratio (Krogh and Schmit-Jensen,

'20) of 50 cal. heat of fermentation per gram mol of methane. The results secured by fitting equation 1 to these adjusted values are given in columns 4 and 5 of table 6, expressed as $100 \div x$ and $100 \div y$, respectively.

The adjusted estimates, somewhat more than the unadjusted estimates, reveal a close similarity in the utilization of the metabolizable energy of the four experimental rations, especially for maintenance. The one discordant value in the general picture presented by the data of table 6, is the low value for body increase secured with ration C. In this connection it may be pointed out that the estimated net availabilities for body increase are probably less accurate than those for maintenance, because most of the metabolizable energy was used for maintenance. This is particularly true for rations C and D at the higher levels of feeding, at which only 11 to 38% of the net energy consumed was stored in the body.

The data of table 6 thus testify strongly to the similar utilization of the metabolizable energy of the four experimental rations, composed of combinations of different feeds but presumably equally well balanced.

SUMMARY AND CONCLUSIONS

It has been shown in metabolism and respiration experiments on eight steer calves of predominantly Shorthorn breeding that the metabolizable energy in four specially designed experimental rations is approximately equally well utilized for maintenance and for body increase. These rations were composed of combinations of different feeds, no one feed occurring in more than one ration, and were so designed both to contain similar proportions of the various classes of nutrients distinguished by proximate chemical analysis, and to be adequate in all essential nutrients.

The similarity in the net availability of the metabolizable energy of rations so constructed lends support to the hypothesis that the extent to which metabolizable energy is utilized for maintenance and tissue synthesis is not a function of the particular feeds included in the ration, but is dependent,

immediately or eventually, on the adequacy of the combination of digestible nutrients thus presented to the tissues in covering their demands for nutriment.

The authors wish to acknowledge gratefully the assistance of other members of the Division of Animal Nutrition in carrying out these arduous and painstaking experiments, particularly the assistance of Dr. F. I. Nakamura in the analysis of the chamber air, and of Mr. Frank Simpson in the care of the experimental animals.

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STUDIES ON NUTRITIONAL ACHROMOTRICHIA IN RATS

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THREE FIGURES

(Received for publication July 16, 1941)

Greying of the fur in black rats maintained on diets deficient in the B complex was first observed by Morgan, Cook and Davison ('38) who showed that this condition could be corrected by the addition of "filtrate factor." Later, Lunde and Kringstad ('39) reported on the efficacy of brewers' yeast, and György, Poling and Subbarow ('40) on the value of liver extracts in curing this condition. With the identification of pantothenic acid as an important constituent of the "filtrate factor," speculation arose as to the effect of this substance on achromotrichia.

The effect of calcium pantothenate on the cure or prevention of greying of the fur in rats maintained on diets free from pantothenic acid has been reported from several laboratories (György and Poling, '40; Unna and Sampson, '40; Emerson and Evans, '41; Unna, '41; Elvehjem, Henderson, Black and Nielsen, '41). On the other hand, R. R. Williams ('40) was unable to demonstrate any effect of pantothenic acid on grey hair in rats, and Frost, Moore and Dann ('41) reported that only few of their animals could be protected from greying by pantothenic acid alone, whereas liver concentrates were much more effective. Recently, Ansbacher ('41) re-

ported greying of the fur in black rats maintained on purified diets supplemented with 500 μg. of calcium pantothenate per rat per day, and that this greying is cured by the addition of para-aminobenzoic acid.

In this communication, data are presented dealing with the effect of calcium pantothenate, liver, rice bran and other substances associated with the vitamin B complex on the achromotrichia in young rats maintained on diets free from pantothenic acid.

The animals used in these experiments were black or piebald rats originating from the Sprague Dawley strain. At 3 weeks of age the rats were placed on a diet having the following percentage composition: dextrose, 68; casein (vitamin free), 18; hydrogenated vegetable oil,[1] 8; salt mixture, U.S.P. XI, no. 1, 4; cod liver oil, 2; supplemented with 0.8 mg. each of thiamine, riboflavin and pyridoxine, 10 mg. of nicotinamide and 100 mg. of choline chloride per 100 gm. of diet. In part of the experiments the dextrose was substituted by sucrose; the hydrogenated vegetable oil by butterfat; and the vitamins of the B complex were added at only half the amount mentioned above. None of these variations produced any significant change in the incidence of achromotrichia.

On this regimen the rats grew at the rate of approximately 1.5 gm. per day throughout the first 3 weeks at which time their weight became stationary and greying of the fur became evident. Simultaneously with the gradual appearance of depigmented hair, the animals developed a scant coarse fur with rusty spots, inflammation of the nose, "blood-caked" whiskers and hemorrhages into the adrenals. These lesions were the same as those occurring in albino rats on a similar diet (Unna, '40 a). The process of greying frequently developed in symmetrical patterns over the body; the hair on the head usually stayed black. Only 50% of the animals survived long enough to develop an iron or silver grey fur extending over the entire body.

[1] Crisco.

Effectiveness of pantothenic acid as compared with liver and rice bran in preventing achromotrichia

In these experiments 3-week-old rats placed on the basal diet received by stomach tube daily supplements of calcium pantothenate in graded quantities ranging from 10 to 100 μg. The growth rate obtained with 25, 40 and 100 μg., as shown in figure 1 agrees closely with the growth responses previously

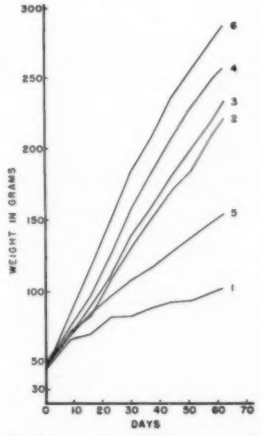

Fig. 1 Effect of daily feeding of calcium pantothenate on the growth of black rats maintained on a diet free from pantothenic acid: 1, no pantothenic acid; 2, 25 μg. of calcium pantothenate; 3, 40 μg. of calcium pantothenate; 4, 100 μg. of calcium pantothenate; 5, 50 mg. of whole dried liver; 6, 250 mg. of whole dried liver.

reported for albino rats (Unna, '40 a). Adrenal hemorrhages which on gross examination were found in approximately 50% of the rats receiving no pantothenic acid, were also observed in rats receiving 10 μg. of calcium pantothenate daily, but not encountered in those receiving 25 μg. As will be noted in figure 1, the growth response to a daily supplement of 250 mg. of whole dried beef liver [2] (containing 75 μg. of pantothenic acid by microbiological assay) was significantly greater than that obtained with 100 μg. of pantothenic acid.

[2] Kindly prepared by Dr. J. C. Keresztesy.

Figure 2 demonstrates the condition of the fur of rats maintained for 25 days on the basal diet supplemented with graded doses of calcium pantothenate or of whole dried beef liver. Rats receiving a daily supplement of 10, 25, and 40 μg. of calcium pantothenate respectively developed a marked greying of the fur, whereas those receiving 100 μg. remained black except for a few scattered grey hairs. A daily supplement of 50 mg. of dried liver, equivalent to 15 μg. of pantothenic acid,

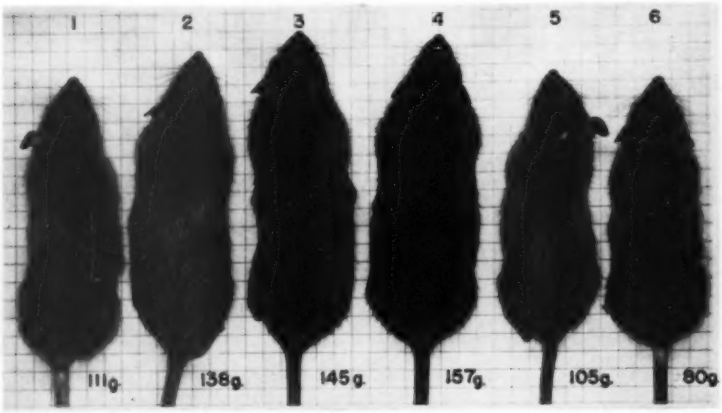

Fig. 2 Condition of the fur of rats maintained for 25 days on the basal diet free from pantothenic acid supplemented with: 1, 25 μg. of calcium pantothenate; 2, 40 μg. of calcium pantothenate; 3, 100 μg. of calcium pantothenate; 4, 250 mg. whole dried beef liver; 5, 50 mg. of whole dried beef liver; 6, no supplement.

did not prevent the achromotrichia, whereas 250 mg., equivalent to about 75 μg. of pantothenic acid, produced an effect equal to that of 100 μg. of calcium pantothenate (fig. 2).

The optimum dose for the prevention of achromotrichia was found to be approximately 100 μg. of calcium pantothenate. Increase of the daily supplement to 250 μg., 500 μg., 1 mg. or 10 mg., did not result in a further improvement in the color of the hair nor in a further gain in weight. Likewise, increasing the liver supplement to 800 mg. produced no improvement over the 250 mg. level.

Rats receiving a daily supplement of 100 μg. of calcium pantothenate have been maintained on the basal diet for more than a year. They remained black throughout the experiment. Litters obtained from these rats were also maintained on the same regimen, and for the most part, the rats of the second and third generation showed a normal black pelt, although in a few instances some scattered grey hairs were observable.

In another series of experiments, the basal diet was supplemented with 10% rice bran (containing 32 μg. of pantothenic acid per gram by microbiological assay). Achromotrichia was regularly obtained on this regimen which, on the basis of a daily food consumption of 10 gm., supplied the rats with a daily amount of about 30 μg. of pantothenic acid. Further supplements of either 50 μg. of calcium pantothenate, 0.2 gm. of dried liver or 0.5 cc. of a rice bran concentrate [3] prevented the achromotrichia.

Curative effect of pantothenic acid as compared with liver

Rats which had been rendered grey after 4 to 7 weeks on the basal diet, were fed daily with calcium pantothenate in amounts from 10 to 500 μg. or with whole dried beef liver. The weight response to the administration of 100 μg. of calcium pantothenate was immediate and continuous. The effect on depigmentation was somewhat delayed. Almost no change in the color of the fur could be noticed during the first week. During the second week, together with a conspicuous improvement in the quality of the hair, new black hair became noticeable, mostly in patterns on the back, on the neck and especially at the root of the tail. The process of restoration of black hair, thereafter, progressed rapidly and was usually complete by the end of the fifth week (fig. 3). Smaller doses of pantothenic acid (25 or 40 μg), although producing an appreciable weight response, did not effect cures of the achromotrichia over a period of 5 to 7 weeks, but occasionally after 8 weeks some scattered black hairs were observed and if the

[3] Nopco.

rats were continued on these supplements for 15 weeks, appreciable darkening of the pelt was noticeable.

The pigmentation of the fur following the administration of whole dried beef liver in amounts of 250 mg. was neither accelerated nor superior to that obtained with calcium pantothenate, although the increase in weight was significantly greater than that following 100 μg. of calcium pantothenate (140 gm. instead of 80 gm. over a 5-week period).

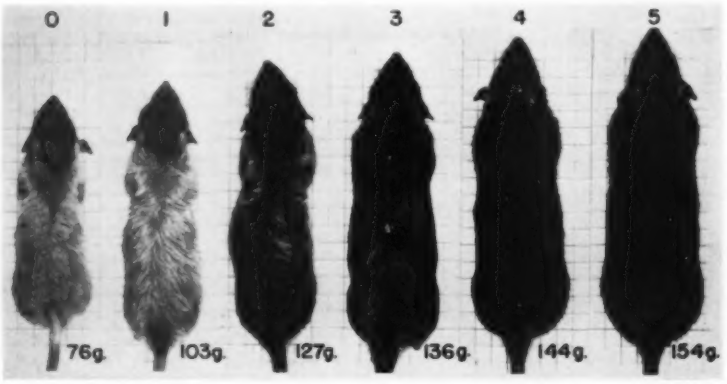

Fig. 3 Effect of daily administration of 100 μg. of calcium pantothenate on achromotrichia: (0) maintained for 22 days on the basal diet before treatment began; (1–5) same rat after 1, 2, 3, 4 and 5 weeks of treatment with pantothenic acid.

Effect of biotin, inositol and para-aminobenzoic acid

It is recognized that the basal diet used in this study is deficient in several factors recently associated with the vitamin B complex other than pantothenic acid. The effect of some of these substances now available in pure form or as concentrates has been studied in rats maintained on the basal diet.

Biotin in the form of the concentrate [4] practically free from pantothenic acid was administered in amounts equal to 0.5 μg.

[4] Concentrate no. 200 (S.M.A. Corp., Chagrin Falls, Ohio); 1 cc. containing 20 μg. of biotin according to microbiological assay.

per day without producing any improvement in achromotrichia or growth. When 100 μg. of calcium pantothenate were given simultaneously with 0.5 μg. of biotin, achromotrichia was prevented; the growth response obtained was not greater than that with pantothenic acid.

In other experiments, the biotin concentrate was administered in the same daily doses after pronounced greying had developed. No effect either on achromotrichia or on growth was observed. Furthermore, the daily feeding of biotin concentrate (equivalent to 0.5 μg.) plus 100 μg. of calcium pantothenate to grey rats did not produce an effect superior to that obtained with calcium pantothenate alone.

Inositol given in daily doses of 3 mg. did not exert any demonstrable effect on rats maintained on the basal diet. When added from the beginning of the dietary regimen, it failed to prevent the achromotrichia. Also, it was ineffective when administered in curative experiments to rats rendered grey on the basal diet.

Para-aminobenzoic acid was administered in daily doses of 3 mg. to rats either from the beginning of the dietary regimen or after they had been rendered grey. No effect was observed in either case; the appearance of grey fur and the growth rate were the same as those of the control rats. Daily administration of 3 mg. of para-aminobenzoic acid over periods extending to 6 weeks were unsuccessful [5] in influencing the scattered grey hairs which frequently persist following the addition of pantothenic acid and which gave these rats a "pepper and salt" appearance.

DISCUSSION

Our results obtained from approximately 2000 black and piebald rats show that young black rats maintained on a highly purified diet free from vitamin B complex but supplemented with thiamine, riboflavin, nicotinic acid, pyridoxine and choline regularly develop conspicuous greying of the fur

[5] Since we failed to obtain greying of the fur in rats receiving adequate amounts of pantothenic acid we were unable to duplicate the report of Ansbacher ('41).

within 3 to 7 weeks. The achromotrichia is associated with the syndrome of pantothenic acid deficiency previously described for albino rats on a similar diet (Unna, '40 a). The occurrence of achromotrichia is not prevented by the addition of calcium pantothenate in doses of 10 to 40 μg. However, a daily supplement of 100 μg. of calcium pantothenate, representing approximately the optimum daily amount for growth on the basal ration described on page 554, prevents the occurrence of grey hair and restores within 4 to 6 weeks the black pigmentation of the fur in rats which have been rendered grey on the deficient diet. Frequently some scattered grey hairs persist in spite of an ample supplement of pantothenic acid. These results are in agreement with previous findings (Unna and Sampson, '40; György and Poling, '40) recently confirmed by Emerson and Evans ('41) and Elvehjem, Henderson, Black and Nielsen ('41). Williams ('40) using a basal diet differing from ours mostly in its low fat content, failed to cure greying of the fur with pantothenic acid. We have conducted experiments using a diet practically free from fat consisting of dextrose, 78%; casein (vitamin free), 18%; salt mixture U.S.P. XI no. 1, 4%; supplemented with 0.8 mg. each of thiamine, riboflavin, and pyridoxine, 10 mg. of nicotinamide and 100 mg. of choline per 100 gm. of diet; in addition the rats received a daily supplement of 100 mg. of linoleic acid concentrate and twice weekly β-carotine (50 μg.) and vitamin D (1000 U.S.P. units) dissolved in 0.15 cc. of ethyl laurate. In this experiment calcium pantothenate was found to be effective in preventing or curing grey hair occurring on this ration, although the growth of young rats on such a diet is poor, and the weight response obtained with pantothenic acid much less marked than in rats maintained on rations containing 10% fat. In our experiments as well as those of Elvehjem et al. ('41), graded responses of repigmentation with graded doses of pantothenic acid were observed, whereas Frost, Moore and Dann ('41) have reported the same incidence of partial cures with 25 μg. as well as with 200 μg. of calcium pantothenate. Also these investigators reported

more pronounced effects on achromotrichia from liver concentrates than from calcium pantothenate.

Black rats have been reared throughout three generations on the basal ration with a daily supplement of 100 μg. of pantothenic acid. No significant achromotrichia has been observed in the offspring. This successful mating of black rats confirms previous findings of Jukes ('40) and of our laboratory on albino rats (Unna, '40 b), recently confirmed by Richardson, Hogan, Long and Itschner ('41).

Biotin, inositol and para-aminobenzoic acid were found ineffective in preventing or curing the achromotrichia in rats maintained on a diet free from pantothenic acid. Emerson and Evans ('41) have likewise reported on the inefficiency of inositol. When pantothenic acid is present in the diet, these substances do not exert an additional effect beyond that of pantothenic acid alone.

Experiments with whole dried beef liver, rice bran and rice bran concentrate on a large number of black rats on our basal ration have failed to demonstrate an anti-grey hair effect of these substances sufficiently superior to that of pantothenic acid to permit postulating the existence of a separate "anti-grey hair factor" (Nielsen, Oleson and Elvehjem, '40; Morgan and Simms, '40). Furthermore, the effectiveness of liver concentrate in restoring pigmentation was destroyed after treatment with alkali. However, the growth-promoting effect of whole dried beef liver was invariably greater than that of equivalent amounts of pantothenic acid.

Pantothenic acid appears to be essential in correcting the depigmentation of the fur occurring on vitamin B complex deficient diets. Since greying is not confined to vitamin B complex deficiencies, but occurs in other conditions such as mineral deficiencies, substances other than pantothenic acid may well be effective under different conditions.

CONCLUSIONS

1. Black and piebald rats maintained on a B complex free diet supplemented with thiamine, riboflavin, nicotinic acid,

pyridoxine and choline develop greying of the fur within 3 to 7 weeks simultaneously with retardation in growth and the appearance of a scant coarse fur, inflammation of the nose, "blood-caked" whiskers and adrenal hemorrhages.

2. Addition of 100 μg. of calcium pantothenate prevents the development of the achromotrichia together with the other deficiency lesions, and restores within 4 to 6 weeks the black pigmentation of the fur in rats which have become grey on the basal diet.

3. The efficacy of liver and rice bran in preventing or curing achromotrichia parallels their content of pantothenic acid, although these materials exert a growth-promoting effect superior to that of pantothenic acid.

4. Black rats maintained on a B complex free diet supplemented with thiamine, riboflavin, nicotinic acid, pyridoxine, choline and pantothenic acid have been raised through three generations.

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INEFFICACY OF HORMONES IN NUTRITIONAL ACHROMOTRICHIA OF RATS[1]

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ONE FIGURE

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Greying of the fur of young black and piebald rats produced by dietary deficiency in pantothenic acid can be prevented by the addition of calcium pantothenate (György and Poling, '40; Unna and Sampson, '40). Also, it has been shown (Unna, Richards and Sampson, '41) that the effectiveness of liver and rice bran depends largely on the amount of pantothenic acid present in such preparations. Morgan and Simms ('40) have reported that the achromotrichia of rats maintained on diets free from "filtrate factor" can be cured by the administration of extracts of the thyroid and of the adrenal cortex. Since pathologic changes have been found in the adrenals of rats maintained on diets deficient in pantothenic acid (Daft and Sebrell, '39; Morgan and Simms, '39; Ashburn, '40), it seemed of interest to study the effect of the administration of hormones to pantothenic acid deficient rats with particular reference to their influence on adrenal hemorrhages and achromotrichia.

Black and piebald rats of a uniform strain were placed at 3 weeks of age on a synthetic diet consisting of dextrose 68%; casein, vitamin free 18%; hydrogenated vegetable oil[2] 8%; salt mixture U.S.P. XI no. 1, 4%; cod liver oil 2%; and supplemented with 0.8 mg. each of thiamine, riboflavin and pyridoxine, 10 mg. of nicotinamide and 100 mg. of choline

[1] Submitted by Charles W. Mushett in partial fulfillment of the requirements for the degree of Master of Science, New York University.

[2] Crisco.

chloride per 100 gm. of diet. In order to facilitate the detection of changes in the hair color, the rats were depilated with barium sulfide at the beginning of the experiment. A thick paste of barium sulfide was rubbed into the fur for about 1 minute, and instantaneous rinsing in warm water removed the hair without causing irritation of the skin. No attempt was made to remove the hair of the head.

In preventive tests, the hormone preparations were administered daily from the beginning of the dietary regime. In each series the number of control and test animals was the same. Weight and symptom records were kept on all animals, and autopsies were performed after death. Curative effects were studied by daily administration of the hormone preparation to rats which had become completely grey after being maintained on the basal diet for at least 4 weeks. In evaluating the efficacy of a hormone, part of a group of grey rats received the hormone, another part an effective dose of calcium pantothenate (100 μg.), and the remainder were left untreated on the basal diet.

Sketches of the distribution of pigment in the skin and of the growth and color of the fur were made twice weekly for every rat on special protocol sheets stamped with a rat's body outline.

The hormone preparations used in this study were: adrenal cortical extract,[3] desoxycorticosterone acetate,[4] thyroid U.S.P.,[5] and anterior pituitary extract.[6] All preparations were found to be free from pantothenic acid by microbioassays on Lactobacillus casei (Snell, Strong and Peterson, '37).

Young black rats on the basal diet without depilatory treatment developed distinct grey patterns within 4 weeks and became entirely grey about the fortieth day. The rats which were depilated at the beginning of the dietary regime, gave evidence of greying in an average of 14 days and were wholly

[3] "Cortin" Roche-Organon—1 cc. equivalent to 50 gm. of fresh adrenal cortex.
[4] "Doca", Roche-Organon.
[5] Lilly.
[6] Squibb.

or mostly covered with grey hair within 24 days (fig. 1). No differences in either growth or the appearance of other symptoms characteristic of pantothenic acid deficiency were observed between these rats and those which had not been depilated. After depilation hair started to grow within 2 to 3 days beginning on the ventral body surface and progressing dorsally up the sides where it met at the midline about two-

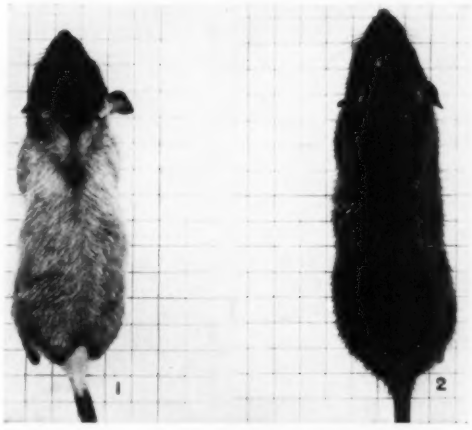

Fig. 1 Greying of the fur after 25 days on a pantothenic acid free diet: 1—Rat depilated at the beginning of the dietary regime showing uniformly grey fur; 2—Rat not depilated showing development of greying in distinct pattern.

thirds anteriorly. From there it progressed caudally and toward the neck. The last areas to become covered were the nape of the neck and the base of the tail. This sequence of hair growth was common to all groups. It coincides with the growth of hair observed by Butcher and Richards ('39) in well-fed albino rats. The wave of hair growth is preceded by the appearance of pigment in the skin and pigmentation of the skin has been observed regardless of whether the new hair comes in black or grey.[7]

[7] Observations on the skin pigment will be reported in another communication.

Prophylactic test

The results obtained with the daily administration of cortical extract, desoxycorticosterone acetate, anterior pituitary extract and thyroid to rats from the beginning of the dietary regime are summarized in table 1. None of the hormones in

TABLE 1

Effect of various hormones on the occurrence of grey hair and adrenal hemorrhages in young black rats maintained on a vitamin B complex free diet supplemented with 0.8 mg. each of thiamine, riboflavin and pyridoxine, 10 mg. of nicotinamide and 100 mg. of choline chloride per 100 gm. of diet.

NO. OF ANIMALS	SUBSTANCE	DAILY DOSE	NO. OF RATS SURVIVING FOR MORE THAN 3 WEEKS	NO. OF RATS SHOWING GREY FUR AT 3 WEEKS	ADRENAL HEMORRHAGE
10	Cortical extract	0.25 cc. intramusc.	6	6	+
5	" "	0.5 cc. "	3	3	+
15	Desoxycorticosterone acetate	2.5 mg. intramusc.	11	10	+
10	Anterior pituitary extract	0.25 cc. intramusc.	3	6	+
10	Anterior pituitary extract	0.5 cc. intramusc.	4	7	+
15	Thyroid U.S.P.	1.0 mg. by mouth	10	8	+
10	" "	5.0 mg. " "	3	3	+
15	" "	10.0 mg. " "	3	4	+
48	Controls	—	35	48	+
45	Calcium pantothenate	100 μg. by mouth	45	0	0

the dosage listed in the table produced any increase in weight of the rats. Rats receiving daily 5 mg. and 10 mg. of thyroid reached an average weight of only 45 and 40 gm. respectively, whereas the average weight at which the other groups and the control animals became stationary was 50 to 70 gm. The mortality of the rats receiving the hormones was somewhat higher than that of the control rats. The administration of thyroid produced toxic effects; seven rats of the group of ten rats receiving 5 mg. of thyroid daily died within 3 weeks, while the same dose of thyroid given daily over a period of

4 weeks was apparently well tolerated by rats maintained on a complete diet. No effect on the rate of growth of new hair after the depilation was observed in rats receiving cortical extract, desoxycorticosterone acetate and anterior pituitary extract. Pigmentation of the skin and growth of new hair occurred somewhat more rapidly in rats receiving thyroid, than in those of the control group. None of the hormones prevented the occurrence of adrenal hemorrhages. All animals surviving for at least 3 weeks showed greying of the fur equal to that of the control rats. On the other hand, the daily administration of 100 μg. of calcium pantothenate prevented adrenal hemorrhages and the depigmentation of the fur.

TABLE 2

Effect of various hormones on rats with grey fur maintained on a vitamin B complex free diet supplemented with 0.8 mg. each of thiamine, riboflavin and pyridoxine, 10 mg. of nicotinamide and 100 mg. of choline chloride per 100 gm. of diet.

NO. OF ANIMALS	SUBSTANCE	DAILY DOSE	DAYS ON BASAL DIET PREVIOUS TO TREATMENT	PERIOD OF TREATMENT IN DAYS	EFFECT ON GREY HAIR
3	Cortical extract	0.25 cc. intramusc.	25	43, 9, 9	None
4	" "	0.5 cc. "	25	43, 42, 42, 22	"
4	" "	0.5 cc. "	33	56, 56, 36, 21	"
4	" "	0.5 cc. "	54	36, 32, 23, 14	"
4	Desoxycorticosterone acetate	0.25 mg. "	45	40, 35, 32, 9	"
4	Desoxycorticosterone acetate	1.0 mg. "	45	32, 32, 32, 14	"
7	Anterior pituitary extract	0.5 cc. "	49	52, 41, 41, 40, 15, 13, 9	"
6	Thyroid, U.S.P.	1.0 mg. by mouth	49	43, 37, 34, 37, 23, 20	"
7	" "	5.0 mg. " "	49	27, 21, 16, 13, 9, 9, 9	"
3	" "	10.0 mg. " "	40	20, 20, 13	"
	Calcium pantothenate	100 μg. by mouth			Black pigmentation restored

Curative test

The results obtained with the daily administration of hormone preparations to rats which had developed grey hair on the basal diet are summarized in table 2. No effect on achromotrichia has been observed on treating the animals with the various preparations over periods extending to 56 days. Adrenal hemorrhages were found in all groups of rats treated with these substances. None of the preparations were capable of prolonging the life of the grey rats. Calcium pantothenate, on the other hand, produced a marked effect on the achromotrichia within 3 weeks, and within 4 to 6 weeks the pigmentation of the fur was practically restored.

DISCUSSION

While the basal diets employed by Morgan and Simms were essentially the same as ours, the method of administering the supplements was different in that we fed all of the supplementary vitamins from the beginning of the test, whereas Morgan and Simms ('40) supplemented their diet first with only thiamine until growth ceased and then added their riboflavin and pyridoxine supplements. This might account for the greater survival time of their animals. It required 56 to 70 days for their rats to begin to show the characteristic changes of the fur. With our procedure, the majority of rats die within 5 to 6 weeks and only a few survive for 10 to 13 weeks. Furthermore, it might be possible, that the thyroid and cortical extracts used by these authors might have contained traces of pantothenic acid.

CONCLUSIONS

The effect of adrenal cortical extract, desoxycorticosterone acetate, thyroid and anterior pituitary extract has been studied on black and piebald rats maintained on a vitamin B complex free diet supplemented with thiamine, riboflavin, nicotinamide, pyridoxine and choline.

1. Daily administration of adrenal cortical extract (0.25 and 0.5 cc.), desoxycorticosterone acetate (2.5 mg.), thyroid

(1, 5 and 10 mg.) and anterior pituitary extract (0.25 and 0.5 cc.) to young rats from the beginning of the dietary regimen failed to prevent the greying of the fur or the occurrence of adrenal hemorrhages and other lesions encountered in rats on diets deficient in pantothenic acid.

2. Daily administration of adrenal cortical extract (0.25 and 0.5 cc.), desoxycorticosterone acetate (0.25 and 1 mg.), thyroid (1, 5 and 10 mg.) and anterior pituitary extract (0.5 cc.) over periods extending to 56 days to rats which had been rendered grey by the dietary regimen, failed to restore the black pigmentation of the fur.

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DIGESTIBILITY AND BIOLOGICAL VALUE OF WHOLE WHEAT BREADS AS COMPARED WITH WHITE BREAD

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ONE FIGURE

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The most ancient and honorable of man's foods, bread is of interest at the present time for what it does not contain of the nutrients from wheat rather than for what it does contain. The complete realization of the fact that white flour lacks both minerals and vitamins contained in the wheat berry has led at last to governmental recommendations calling for restoration of the lost substances. Thus far, however, no emphasis has been placed on the relative values of the proteins contained in the entire grain as compared with the endosperm alone. The evidence is not adequate.

Klein, Harrow et al. ('26) made a study on rats of the nutritive value of the various layers of both wheat and corn. It was already known that the various layers of the cereal grains differed not only in their vitamin content and in their content of inorganic salts, but also in their general protein make-up. These authors found that the fractions which contain the pericarp and the germ, and which serve as the distinguishing ingredients of the dietetically superior whole grain flour, possess a much higher nutritive value for rats than fractions which are composed mainly of the endosperm. The milling fraction known as "red dog" gave the highest biological value of any of the fractions studied.

Boas-Fixsen and Jackson ('32) found that proteins of ground whole wheat of the soft Red English variety had by the nitrogen balance method a biological value of 68 in rats, when fed at the level of 6% protein. Wheat germ and endosperm of the same wheat fed at 7% level gave B.V.'s of 69 and 61. In 1934 the same workers with J. C. D. Hutchinson studied the protein values of the same whole wheat and whole yellow maize (from South America) fed at near 10% level, by the rat growth method, and found the rate of growth for the former to be 1.36 gm. per gram of protein fed, and for the latter 1.29 gm. When suitable deduction was made for the protein required for maintenance the values for growth alone were 1.85 and 1.73. Wheat apparently is slightly better than maize as a source of protein both for growth and maintenance of the rat. That the level of protein is very important is shown by the further work of Chick, Boas-Fixsen, Hutchinson and Jackson ('35). Whole wheat at 3.6% level gave 100% B.V., and at 5.6% level only 68; wheat germ at 3.6% level gave 90%, and at 6.8% level 69.

There is only one study available on biological values of breads in the modern sense, namely, the very excellent short paper of French and Mattill ('35). In this study the biological values for the proteins of white bread, "whole wheat" (50-50, meaning 50% of white flour and 50% whole wheat) and of rye bread (25-75) were determined both on rats by Mitchell's method and on human subjects in comparison with whole milk as the standard protein. On mature rats white bread protein had a biological value of 83, "whole wheat" 80, and rye bread 82. The average milk replacement value for six human subjects on the same breads was exactly the same for all the breads, namely, 83. Milk itself, however, has a biological value of about 83 to 86 (Boas-Fixsen, '34–'35).

It seemed worth while, therefore, to make a new study of the biological value of some truly whole wheat breads and of white breads by using whole egg as a standard protein, a method previously used in this laboratory (Murlin, Nasset and Marsh, '38) for the study of cereal breakfast foods on human subjects. Whole egg proteins fed at a 5% level of

intake have given the highest biological value [1] of any known human food when fed to adult rats (Mitchell and Carman, '24; Sumner, '38). The occasion for such a study came with the invention of a new method of removing the rough outer part of the bran from wheat—the flotation process of Earle.[2]

TABLE 1

The diet squad and calorie distribution of diet.

GROUP a [1]	AGE	HEIGHT	SURF. AREA	BEG. WEIGHT	WEIGHT CHANGE	CALORIES			
						Total intake	Distribution		
							P.	CHO	F.
		cm.	*sq. M.*	*kg.*	*kg.*		%	%	%
1. R.R.	27	167.5	1.78[1]	68.8[1]	—0.8	2969	4.8	49.2	46.0
2. T.H.L.	34	162.5	1.57[2]	54.5[2]	—3.4	2453	4.6	48.0	47.4
3. D.F.	25	182.9	1.85[3]	65.2[3]	—2.0	2731	4.9	47.9	47.2
4. J.P.L.	26	182.3	2.03[4]	81.3[4]	—2.3	3822	4.4	48.0	47.6
5. D.H.B.	29	177.8	1.96[5]	76.9[5]	—0.1	2982	5.3	48.0	46.7
GROUP b [1]									
6. C.D.K.	32	170.2	1.78[1]	67.5[1]	+0.9	2834	5.0	48.0	47.0
7. E.S.N.	41	171.5	1.90[5]	77.6[5]	—0.3	3021	5.3	47.9	46.8
8. R.A.B.	24	190.0	2.04[4]	79.9[4]	+0.8	3561	4.6	50.4	45.0
10. F.L.T.	25	163.0	1.62[2]	58.0[2]	0.0	2415	4.9	50.5	44.6
11. L.E.E.	26	177.8	1.85[3]	68.5[3]	—0.2	3151	4.5	48.0	47.5
Average	29	176.7	1.84	69.8	—0.8	2914	4.8	48.6	46.6

[1] Note that subjects in the two groups *a* and *b* may be paired well, as indicated by small index figures, for weights and surface area.

For the determination of biological value of the proteins and their over-all digestibility human subjects were chosen because of the direct applicability of the results to current

[1] Biological values for whole egg proteins have not been determined for adult men by the most modern methods. That it is decidedly superior to milk was shown by Sumner, Pierce and Murlin ('38).

[2] U. S. patent No. 2,143,306. This process removes only the thin epidermis of the wheat berry, leaving intact in the "peeled wheat" all nutrients of the wheat grain available to man. At the invitation of the Continental Baking Company, which controls the Earle patents on this process, the experiment to be described was begun on January 14th and was concluded on March 27th, 1941. The entire study contemplated a complete assay of the B-complex vitamins, and studies also on the relative rates of digestion of the different kinds of bread, which will be reported later. The invitation was accepted in the belief that the information to be gained would be of timely importance nationally.

national problems in nutrition. The squad consisted at the start of eleven men, all apparently in good health, but one was obliged to withdraw at the end of the eighth period because he felt that the diet did not sufficiently maintain the vigor of body and mind necessary to keep up his work. All were members of the institution as graduate or medical students or as members of the departmental staff. Table 1 describes their physical characteristics, their individual weight changes during the experiment, the total calorie intake, and the distribution of calories chosen, within a narrow freedom of choice, by each person.

The diet was planned to supply, as nearly as possible, 80% of the food nitrogen from whole egg, as standard protein, and 80% from bread in alternate periods of 6 days each. Approximately 10% was contained in butter and cream,[3] and 10% in the fruits, vegetables, and accessories as indicated in the sample diet shown in table 2. Also illustrated are the amounts of each constituent, the nitrogen supplied by each, the grams carbohydrate, and the total calories. The choice of a rather high fat diet was dictated by the necessity of supplying sufficient energy without excessive bulk or too much sweetness. The protein level was kept at, or very near, 5% of the total energy, on the well known principle that the body makes more economical use of protein the lower the supply. No subject varied at any time from his average of protein more than a few tenths of 1%, or from his average for carbohydrate and fat more than 2% or 3%. There were a few minor changes

[3] The 4x cream was obtained by direct daily delivery from the same herd of cows on the Markham-Puffer farm near Avon, New York. Eggs were obtained also by frequent delivery from the same flock of hens fed throughout on the same feed. The butter was obtained from the Beatrice Creamery Company, and kept in the Hospital refrigerated storage room. The applesauce was obtained from the W. N. Clark Canning Company, and represented a fixed mixture of New York-State-grown Twenty Ounce, Greenings, and Baldwins. Orange juice was expressed daily from oranges, one-half of which were purchased locally and one-half donated through the local representative of the California Fruit Growers' Association. Lettuce was the iceberg variety of varied import. Coffee was Chase and Sanborn brand. It will be observed that no account is taken of the coffee nitrogen as intake because in the experience of this laboratory it is all secreted by the kidneys of healthy men. It was, therefore, deducted from the daily nitrogen output in the urine.

in total calories to keep weight changes low, but adjustments were always made as to percentage distribution.

The breads chosen for comparison were: (1) a well known breakfast cereal[4] which, while not a bread in the strictest sense, is, nevertheless, a truly whole wheat "baked" product; (2) a local "100% whole wheat bread" which, it was later found, contained 5% of non-fat milk solids from buttermilk

TABLE 2

Sample diet (C.D.K., net weight 67.5 kg.).

EGG—PERIOD III	Gm.	N	CHO	Cal.	LEAN WHITE BREAD—PERIOD IV	Gm.	N	CHO	Cal.
Egg [1]	222	4.51		333.1	Bread,				
4x cream	200	.551	5.8	722.9	lean white	314	4.68	169.1	820
Butter	24	.024		188.8	Butter	62	.062		476.8
French dressing	9	.005	.3	61.7	4x cream	183	.586	5.3	659.9
Lettuce	60	.08	1.8	10.8	Lettuce	120	.16	3.6	21.6
Applesauce	200	.04	48	196	French				
Orange juice	296	.326	38.6	166	dressing	30	.015	.8	205.5
Karo	26	.005	20	80	Applesauce	300	.06	72	294
Sugar	75		75	300	Orange juice	318	.35	41.4	165
Corn starch					Karo	33	.01	25	100
biscuit [2]	3X [3]	.09	151	774	Sugar	23		23	92
Coffee	3X				Coffee	3X			
		5.63	340.5	2833.3			5.92	340.2	2834.8
Protein		5%			Protein		4.86%		
Fat		47.0			Fat		47.1		
Carbohydrate		48.1			Carbohydrate		48.0		

[1] Eaten principally as whole egg plain omelet.

[2] Contained a small amount of the egg allowance.

[3] Times daily.

based upon the flour weight; (3) the whole wheat bread baked[5] from the "peeled wheat" flour (see footnote 2, p. 575); (4 and 7) two lean white breads differing decidedly in their analyses purchased from local chain stores; (5) the whole wheat bread baked[5] from "peeled wheat" with high vitamin yeast,[6] and (6) a white bread containing 5% non-fat milk solids based upon the flour; and made with high vitamin yeast.[6]

[4] Shredded Wheat Biscuit.

[5] By the Continental Baking Company.

[6] High vitamin yeast, made by the Fleischmann Laboratories, contained the following per pound of the baker's yeast in the moist state: Iron 9.1 mg., nicotinic acid 82 to 103 mg., thiamine approximately 90 mg., riboflavin 11.2 mg. It was used in the amount of 3.5 pounds per 100 pounds of the flours.

RESULTS

The data necessary from each subject and their progressive use in calculation of true digestibility and biological value of the proteins in one of the breads are illustrated in table 3. Urines were analyzed only for the last 4 days of each period. Feces were separated for entire 6-day periods by means of charcoal markers taken at the first meal of each, and by means of the characteristic colors and textures produced by the test foods. All foods containing nitrogen were sampled each day, the samples pooled, and aliquots analyzed by periods.

Bread fecal nitrogen minus the average egg fecal nitrogen for each subject gives the fecal food nitrogen (or, otherwise, excess "alimentary nitrogen") for that bread, on the assumption that no egg nitrogen as such reaches the feces. This assumption is not completely borne out by the facts, for occasionally traces of characteristically colored whole egg were seen in the feces, but it is justified by the consistency of the figures for the egg fecal nitrogen for each subject in all the seven periods and by the fact that these figures agree very closely with those in white bread feces, which notoriously contains minimal food nitrogen.

Subtracting fecal food nitrogen from the total nitrogen intake of the bread diet for each subject gives the absorbed nitrogen, and the percentage which this represents of the total is the true digestibility of the protein being studied, other constituents of the diet remaining constant. The slight variations from egg to bread period in the low-nitrogen constituents, made necessary to keep percentages of protein, fat, and carbohydrate equal, are illustrated in table 2.

Biological value of the bread proteins is determined by the excess of the urine nitrogen of the bread period over that of the egg periods, expressed as a percentage of the absorbed nitrogen and subtracted from 100. Table 3 shows that the average B.V. of the local "100% whole wheat bread" which contained 5% non-fat milk solids is 77.8 with a mean deviation for the ten subjects of ± 5.5, and standard deviation of 6.51.

TABLE 3

Biological value of local "100% whole wheat bread" + 5% non-fat milk solids (based on daily averages).

	SUBJECT	a BREAD FECAL N	b AV. EGG FECAL N	c a − b FECAL FOOD N	d TOTAL FOOD N	e ABSORBED FOOD N	f TRUE DIGESTIBILITY ABS. N × 100 / TOTAL FOOD N	g AV. BREAD URINE N	h AV. EGG URINE N	i g − h EXCESS URINE N	j EXCESS N × 100 / ABSORBED N	k B.V. 100 − j	DEV. FROM MEAN
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	%	*gm.*	*gm.*	*gm.*	%	%	%
Period II	1	1.70	0.99	0.71	5.79	5.08	87.7	5.85	4.46	1.39	27.4	72.6	—5.2
	2	1.19	0.83	0.36	4.58	4.22	92.1	4.29	3.35	.94	22.3	77.7	—0.1
	3	1.45	1.10	0.35	5.47	5.12	93.6	6.20	4.53	1.67	32.6	67.4	—10.4
	4	1.86	1.23	0.63	6.89	6.26	90.8	7.43	5.48	1.95	31.1	68.9	—8.9
	5	1.28	1.08	0.20	6.41	6.21	96.9	6.21	4.65	1.56	25.1	74.9	—2.9
Period X	6	1.41	1.02	0.39	5.98	5.59	93.5	5.29	4.11	1.18	21.1	78.9	+1.1
	7	1.30	1.04	0.26	6.88	6.62	96.2	6.14	4.96	1.18	17.8	82.2	+4.4
	8	1.49	1.00	0.49	7.06	6.57	93.0	6.23	5.42	0.81	12.3	87.7	+9.9
	10	1.19	0.71	0.48	5.10	4.62	90.6	4.49	3.68	0.81	17.5	82.5	+4.7
	11	1.38	1.00	0.38	6.08	5.70	93.7	5.22	4.40	0.82	14.4	85.6	+7.8
		1.42	1.00	0.42	6.02	5.60	92.8	5.73	4.50	1.23	22.2	77.8	Av. ±5.5
													St. D. = 6.51

It was necessary in this study to use the average fecal and urine nitrogens for all the egg periods instead of for the two egg periods adjacent to each bread period, for the reason illustrated in figure 1. The average egg urine nitrogen for the entire squad declined steadily from the first period to the last. There was no compensating drift in the tendency of

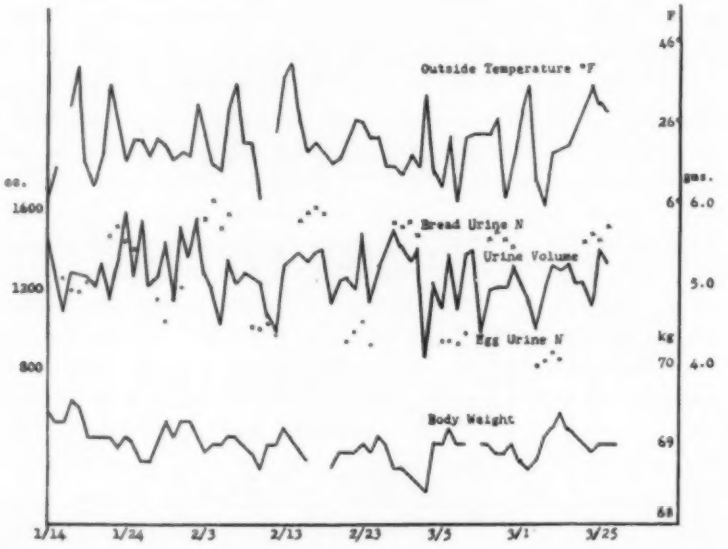

Fig. 1 Graphic comparison of average urine volumes with outside temperature during the course of the experiment. There is no close correlation. The average body weight is shown to increase in egg periods and to decrease in bread periods. There is a progressive decrease in average egg urine nitrogen (dots) with no compensatory change in bread periods (crosses).

the bread proteins. Hence, to compare a bread at the beginning of the series with its associated egg periods would have given the first bread a considerable advantage over the last. This phenomenon of a steady increase in retention of egg protein was not seen in the study of cereal proteins reported in 1938. That study was carried out in late spring and early summer, while this one covered the coldest part of the winter.

No close correlation, however, is to be made out (fig. 1) between outside temperature and either the urinary nitrogen or the urine volume. We are at a loss to explain the difference in the two experiments unless the gradually accumulating deficit of high-value protein caused by a longer and longer dependence on cereal protein for maintenance was greater in the present instance because whole grain products were used in a smaller percentage of the periods.[7]

Average essential data and the computed digestibilities and biological values for all seven of the breads are given in table 4. Only four of the breads, however, namely three whole-wheat and one lean white loaf were eaten by all ten of the subjects. Only these four, therefore, are strictly comparable. The comparative digestibility is indicated by the fecal food nitrogen in column c. The roughest of the whole wheat products, the whole wheat cereal biscuit, gives the highest fecal food nitrogen, and therefore the lowest absorption. Since the average food N was substantially the same for all the breads, this produces also the lowest true digestibility.

The two whole wheat true breads differed in two respects. The local bread contained all of the bran and also 5% non-fat milk solids on the basis of dry flour and added as buttermilk. The new bread lacked the epidermis of the wheat, constituting about 2% by weight of the grain, but no milk solids. It is a little surprising, therefore, that the average fecal food nitrogen is exactly the same for the two, and the consequent true digestibility the same. Apparently the removal of the fibrous outer epidermis in the one has compensated for the digestibility advantage of the added 5% milk solids in the other. As would be expected, the lean white loaf produced the lowest fecal food nitrogen and the highest digestibility.

Some of our foremost nutritional authorities have fallen into the fallacy that highest digestibility of protein confers

[7] The percentage levels of the egg protein calories were nearly identical in the two studies. There were, however, in the former studies some larger additions to total calorie intake in later periods which may have influenced the N balances slightly.

TABLE 4

Average digestibilities and biological values of seven breads (based on daily averages).

NO. AND NAME OF BREAD	NO. OF SUBJECTS	a BREAD FECAL N	c FECAL FOOD N	d TOTAL FOOD N	e (d − c) ABS'D N	f $\frac{e \times 100}{d}$ TRUE DIGESTIBILITY	g BREAD URINE N	i EXCESS N	j $\frac{i \times 100}{e}$ %	k B.V. 100 − j	l ST. DEV.	REMARKS
		gm.	*gm.*	*gm.*	*gm.*	%	*gm.*	*gm.*	%	%		
1. Whole wheat cereal biscuit [1]	10	1.89	0.89	6.00	5.12	85.2	5.46	0.96	18.4	81.6	8.5	
2. Whole wheat bread, 5% non-fat milk solids	10	1.42	0.42	6.02	5.60	92.8	5.73	1.23	22.2	77.8	6.5	
3. Peeled wheat bread	10	1.42	0.42	6.06	5.64	93.0	5.65	1.15	20.4	79.6	4.1	Extra B vitamins, see text, p. 584.
4. Lean white bread I	10	1.03	0.03	6.09	6.06	99.4	6.00	1.50	24.7	75.3	4.4	Same extra B vitamins, see text, p. 584.
3. Peeled wheat (ordinary yeast)	5 (a)	1.34	0.30	6.03	5.73	94.9	5.77	1.28	22.1	77.9	5.8	No extra B vitamins
5. Peeled wheat (high vit. yeast)	5 (b)	1.27	0.32	6.11	5.79	93.6	5.62	1.11	19.0	81.0	5.6	See footnote 6, p. 577
6. White bread (with high vit. yeast and non-fat milk solids)	5 (a)	1.11	0.06	5.84	5.78	98.9	5.68	1.19	20.2	79.8	3.7	See footnote 6, p. 577
7. Lean white bread II	5 (b)	1.07	0.11	6.12	6.03	98.3	6.04	1.53	25.3	74.6	3.9	No extra B vitamins

[1] Shredded Wheat of National Biscuit Company.

greatest nutritive value. It is evident from the table that of the four breads now being considered the one with lowest digestibility produced the highest B.V. It must be remembered that the same average egg urine nitrogen (column h, table 3) was subtracted from the several bread urine nitrogens in table 3 to get excess nitrogen. Thus comparing the two whole wheat breads eaten by ten subjects, we find that one gave a slightly higher absorbed N but produced slightly less urine nitrogen, and therefore showed a higher B.V. The difference is not statistically significant, but it illustrates the fact that a lesser absorption may be succeeded by a greater retention which in reality gauges B.V. The fact that one of these whole wheat breads contained the added milk protein might explain why the B.V. of the other, lacking the epidermis of the grain, was not significantly greater.

The second whole wheat bread (no. 3 of table 4) in comparison with the lean white loaf I shows 0.42 gm. less absorption but shows only 0.35 gm. less urine N, and yet the percentage of the absorbed N which the excess N represents is, for the white bread, over 4.3% greater than for the whole wheat—a difference which, reflected in the B.V., is statistically significant. The difference between the B.V.'s of the whole wheat biscuit and lean white bread also is statistically significant, but not the difference between those of the local whole wheat bread and the lean white even though the former carried 5% milk solids.

The last four comparisons in table 4 were made on only five subjects for each bread. The subgroup *a* contained the first five subjects and subgroup *b* the second five listed in tables 1 and 3. The object of these periods was to get some light on the influence of "high vitamin yeast"[8] compared with ordinary yeast in the baking of a whole wheat bread and of white bread. The first comparison indicates that the high vitamin yeast in the peeled wheat bread conferred a higher B.V. than the same bread with ordinary yeast. We have no evidence that one of the subgroups regularly produced a

[8] See footnote 6, page 577.

higher B.V. than the other. Furthermore, in comparing different sources of the B-complex (see below) the source[9] which proved superior with one group did so with the other.

The white bread containing the high vitamin yeast also gave a higher biological value than the lean white loaf containing ordinary yeast, and the difference is great enough to be statistically significant for a larger number of subjects. The white bread baked with high vitamin yeast, however, contained also 5% non-fat milk solids, and this constituent may have added to its biological value. It is well known (Mitchell and Carman, '26) that milk and white flour proteins do supplement each other when fed together in the proportion of 1 to 2. The proportion of milk to flour proteins in the bread is much lower than this and therefore the exact effect of its milk protein can only be conjectured. More work should be done on these supplementary effects.

Effect of extra vitamins on biological value. An attempt was made to learn whether extra B vitamins added to a diet already approximately adequate in these factors would improve the B.V. of the cereal proteins. Accordingly in the third bread period when one of the lean white breads and the peeled wheat bread were eaten by groups *a* and *b* respectively, a quantity of a certain concentrate[10] sufficient to supply 750 I.U. of thiamine, 0.9 mg. of riboflavin, 300 μg. of pyridoxine, 30 rat growth units of filtrate factors and nicotinic acid equivalent to 37 gm. fresh whole liver was given to each man of both subgroups for 4 of the 6 days. For the last 2 days the vitamin concentrate was a different one,[9] the daily dose containing approximately 500 I.U. of thiamine, 125 Sherman units of riboflavin and other members of the B-complex from yeast.

In the following egg period and the fourth bread period when the same breads now were eaten by groups *b* and *a* respectively (the reverse of bread period 3) extra vitamins were continued in approximately one-half the amount (i.e.,

[9] Harris' "Yeast Vitamine", 7½ grain tablets.

[10] Lederle's "Vitamin B complex, oral".

250 I.U. of B_1 and 62 Sherman units of B_2). The two groups in any period were receiving the same extra vitamins. It is of interest that in these two periods combined the B.V. of the peeled wheat bread was higher than when the same bread (second peeled wheat bread 3 in table 4) was eaten without extra vitamins. Also the B.V. of the lean white bread 4 eaten with extra vitamins was higher than that of lean white bread 7 eaten without extra vitamins. The difference in neither case is statistically significant, but probably would prove to be so with more subjects.

Economic considerations. In 1918 a report was issued by a committee of the Royal Society of London entitled "The Digestibility of Breads", from which it was concluded on the basis of the yearly consumption of wheat in the United Kingdom in the years immediately preceding the first world war, that if all of the population had eaten the bread made from 90% extraction flour instead of the bread made from 80% extraction flour there would have been a yearly saving of calories equal to an extra month's supply for the whole United Kingdom.

The saving from the use of the peeled wheat bread used in this study as compared with the lean white bread I is indicated in table 5.

From the comparison shown in this table it is evident that the saving would amount to 93,737 kg.-calories, or enough to support the average person (2200 cal.) for over 40 days.

But this is not the entire advantage of whole wheat. If the average person in the United States who now eats lean white bread were to change to an acceptable whole wheat bread such as the peeled wheat bread which was the nearest to 100% of any true bread used in this investigation, there would result not only this large saving in calories per person in the country's human food supply, but in addition the consumer would get the entire B-complex of vitamins contained in wheat, more minerals, especially calcium, phosphorus and iron, and his tissues would have the advantage of a biological

value of the protein significantly higher than that of the lean white bread.

These are considerations too important to be overlooked. The country is awake to the importance of better nutrition; it should be awake also to the importance of vital economy in nutrition.

TABLE 5

Computation on calorie basis of saving from use of whole wheat.[1]

	PEELED WHEAT BREAD	LEAN WHITE BREAD
Composition as eaten.		
N = 1.69%, Protein 9.633%		1.43% Protein 8.125%
Carbohaydrate (by hydrolysis)	38.4%	48.0%
Fat (petroleum ether extract)	3.21%	2.6%
Digestibility.		
Protein	93.0%	99.4%
Carbohydrate	98.6%	99.7%
Fat	97.1%	97.4%
Computation of calories.		
Protein	9.633 × .93 × 4.1 = 36.73	8.151 × .994 × 4.1 = 33.22
Carbohydrate	38.4 × .986 × 4.1 = 155.22	48.0 × .997 × 4.1 = 196.18
Fat	3.21 × .971 × 9.3 = 28.99	2.6 × .974 × 9.3 = 23.55
Total per 100 gm.	220.94	252.95
Calories per pound	1003.1	1148.4
100 pounds wheat produce	98 pounds flour	69.4 pounds flour
98 pounds flour produce	158 pounds bread	104 pounds bread
158 × 1003.1 =	158.490 calories	119.433 calories

Taking 240 pounds (4 bushels) as average per capita consumption,[2]
2.4 (158.490 — 119.433) = 93.737 calories saved.

[1] The two breads chosen are the ones used in this investigation which were eaten by the entire squad and showed the greatest contrast in nutrient qualities.

[2] A recent bulletin (mimeographed) from the U. S. Department of Agriculture entitled "Consumption of Agricultural Products" gives the average per capita consumption of flour, for the 10 years 1930–1939, as pounds of wheat, at 226.

SUMMARY AND CONCLUSIONS

Digestibilities and biological values of the proteins of whole wheat and white breads were determined on ten healthy male subjects averaging 29 years of age.

Average daily intake of energy was 2914 kg. cal., distributed, with slight variations, 4.8% to protein, 48.6% to carbohydrate and 46.6% to fat.

Six-day periods on egg as the source of 80% of the N alternated with like periods on bread as the source of this fraction. Ten per cent of the N in all periods came from cream and butter and 10% from fruits, vegetables, and accessories. Twenty-four-hour urines were analysed on the last 4 days, feces in one lot for the full period.

Three whole wheat breads and one whole wheat cereal biscuit were compared with three white breads. In general the whole wheat breads gave lower true digestibility values for the protein than the white, but at the same time produced higher biological values. One white bread containing 5% non-fat milk solids produced a higher B.V. than a whole wheat with the same milk content. Taking the B.V. of egg proteins as 100% the B.V.'s for the proteins of the four whole wheat breads were: whole wheat cereal biscuit, 81.6; a locally made whole wheat bread containing 5% non-fat milk solids, 77.8; "peeled wheat" bread eaten with extra B vitamins, 79.6; same baked with high vitamin yeast, no other extra vitamins, 81.0; same with ordinary yeast, no extra B vitamins, 77.9. For the white breads: lean white I eaten with the same extra B vitamins as with the "peeled wheat" bread, 75.3; similar bread II eaten with no extra B vitamins, 74.6; white bread containing 5% non-fat milk solids and baked with high vitamin yeast, eaten with no extra B vitamins, 79.8.

The results suggest that making bread with "high vitamin" yeast is of some importance from the standpoint of the biological value of proteins; also that eating extra B-complex vitamins improves biological values.

The authors are grateful to Dr. E. S. Nasset and Mrs. Robert Ryer, III, for assistance at important points in this investigation.

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APPARENT DIGESTIBILITY OF CARBOHYDRATES, FATS, AND "INDIGESTIBLE RESIDUE" IN WHOLE WHEAT AND WHITE BREADS

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ONE FIGURE

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An investigation of the digestibility and biological value of the proteins of white, whole wheat, and peeled wheat breads has been reported in the preceding paper (Murlin, Marshall and Kochakian, '41). It is important that the comparative availability of other nutrients in these breads be established. This is particularly pertinent in view of the present discussion relative to enrichment of flours and breads with some of the B vitamins, and the alternative program of the use of higher and higher extraction flours, and/or higher vitamin yeasts.

Accordingly, we have studied from the material available in the preceding investigation the apparent digestibility of carbohydrate, fat, and the "indigestible residue." Since one of the chief differences between white and whole wheat breads is the higher content of roughage, or "indigestible residue" of the latter, particular attention has been paid to this component.

EXPERIMENTAL

For the plan of the experiment, the composition of the diet and other aspects, the reader is referred to the preceding paper.

Except in those instances noted in the summary table the various food items were analyzed after drying and grinding.

Sufficient numbers of samples were taken throughout the duration of the experiment so that the final value represents a reasonable average for the entire experiment. Analysis of the feces was made from alcohol-dried aliquots that were thoroughly pulverized for sampling.

Ash was determined by brief combustion at 600°C. in the muffle furnace. In order to determine total carbohydrate, both food and feces samples were refluxed 2 hours in 2% hydrochloric acid. The neutralized hydrolysates were precipitated with zinc hydroxide according to the method of Somogyi ('30). Aliquots were then analyzed by the copper-iodometric method of Shaffer and Somogyi ('33), the final calculations being made from a glucose calibration curve. Admittedly glucose calibration is inadequate for a diet containing mixed carbohydrates; however, for purpose of the comparison desired in this experiment this procedure has proved satisfactory. The fat of the foods and feces was determined as the total petroleum ether soluble material by the wet extraction method of Saxon ('14). In order to insure complete extraction of free fatty acids, hydrochloric acid was added as directed in the original method. Additional aliquots were analyzed for indigestible residues by the fractional method of Williams and Olmsted ('35), the final value being obtained by the addition of the lignin, hemicellulose and cellulose found.

The authors are quite aware of the theoretical limitations attendant upon the use of each of these procedures in given instances. However, in view of the fact that final choice was made on the basis of convenience of the procedure, the particular information desired and other circumstances relating to the conduct of the experiment as a whole, the results should prove entirely adequate.

RESULTS

The items used in the diet experiment are listed in table 1 with the composition found by the methods described. The list of breads is identical with the list given in table 4 of the previous paper. The higher ash content and lower carbo-

TABLE 1

Composition of food items. Per cent of dry weight except where indicated.

FOOD	DRY WEIGHT	ASH	CARBO-HYDRATE	FAT	PRO-TEIN[1]	INDIGESTIBLE RESIDUE			
						Lignin	Hemi-cellulose	Cellu-lose	Total
	%								
Whole wheat cereal biscuit[2]	93.80	1.89	70.20	1.46	11.30	0.900	4.200	4.290	9.39
Locally made whole wheat bread, 5% non-fat milk solids	61.60	3.09	64.40	2.89	15.70	0.887	3.540	2.740	7.17
Peeled wheat bread	62.60	3.57	61.10	5.12	15.50	0.930	3.850	2.990	7.77
Lean white bread I	68.90	2.71	69.70	3.79	11.70	0.083	0.908	1.510	2.50
Peeled wheat bread with high vitamin yeast	67.90	3.27	52.30	5.14	14.40	0.815	4.850	2.610	8.28
White bread, 5% non-fat milk solids, high vitamin yeast	68.60	2.80	61.80	4.57	12.00	0.045	0.727	1.980	2.75
Lean white bread II	67.60	2.64	72.00	3.33	12.50	0.115	1.530	0.950	2.60
Eggs	26.85	3.45	1.95	43.20	48.30				
Applesauce	21.68	0.69	33.50		0.56	0.780	2.060	1.820	4.66
Butter[3]		1.72	1.10	78.27	0.61				
Cream, 4X[3]		0.41	1.83	40.26	1.91				
Corn syrup	75.50	0.92	88.70		0.17				
Lettuce	4.15	12.33	19.40	1.80	7.56	3.970	7.250	8.800	20.02
Orange juice	10.40	4.42	61.90		0.81	0.212	0.972	0.625	1.82
French dressing[3]				25.00	0.31				
Cornstarch biscuit		3.55	86.40	10.10	0.52				

[1] N × 5.7 for cereal products: N × 6.25 for all others.

[2] Shredded Wheat Biscuit of National Biscuit Company.

[3] Values recorded on fresh basis.

hydrate of the whole wheat breads as compared with the lean white breads are, of course, according to expectation. As ordinarily given in tables of composition the carbohydrate is obtained by difference after adding together the ash, protein, fat, and fiber, and subtracting these from the total dry weight. The values obtained by hydrolysis as noted above are naturally considerably lower for the carbohydrate than those found in most food tables.

The protein content of the cereal products was obtained from the nitrogen by use of the factor 5.7, as is the more recent custom among cereal chemists. All other protein values in table 1 were obtained by using the more familiar factor, 6.25. It is of special interest that all of the whole wheat breads show higher protein content than the white breads, with the exception of the whole wheat cereal biscuit,[1] which is included in this study, as in the previous one, not because it is a bread in the strict sense of the word, but because it represents a truly whole wheat "baked" product. The highest value for protein in this table is the locally made whole wheat bread, which contained 5% non-fat milk solids on the basis of dry flour.

Of particular interest at this time are the values for "indigestible residues" by the fractional method. As pointed out by Williams and Olmsted, the values by this method are considerably higher for all foods containing fiber than those obtained by the old Weende procedure. This difference is quite apparent, for example, in the case of the peeled wheat bread and the locally made whole wheat bread. The former on the dry basis by the fractional method gives a total indigestible residue of 7.7%, whereas with the older procedure the value reported is 1.7% crude fiber. The locally made whole wheat bread shows respective values of 7.17% and 2.04%.

In table 2 is reported the average composition of the feces for the different periods. Since the diet was so designed that the supplementary food items remained comparatively constant throughout, the differences recorded are directly resultant from the test food of a particular period.

By deducting the figures of table 2 from the total intake the per cent utilization (in the older sense), or apparent digestibility (in the newer) has been calculated as shown in table 3. It is of interest that although there is considerable variation in the per cent of mineral absorbed (lean white II only 62.1%, others varied from 70.5 to 84.4%), with the exception of the egg and whole wheat cereal biscuit periods

[1] Shredded Wheat Biscuit of the National Biscuit Company.

TABLE 2

Analysis of feces. Average values for 6 day periods.

EXPERIMENTAL FOOD	NO. OF SUBJECTS	TOTAL WEIGHT	SOLIDS	ASH	CARBOHYDRATE	FAT	INDIGESTIBLE RESIDUE			
							Lignin	Hemicellulose	Cellulose	Total
		gm.	%	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Egg	10	447	25.8	14.2	4.7	26.6	5.45	4.67	3.66	13.78
Whole wheat cereal biscuit [1]	10	1102	24.3	28.8	38.0	38.0	19.10	43.20	18.00	80.30
Locally made whole wheat bread, 5% non-fat milk solids	10	731	25.8	18.6	20.0	24.5	16.90	22.60	11.30	50.80
Peeled wheat bread [2]	10	646	28.0	18.2	15.8	27.0	14.30	19.30	10.70	44.30
Lean white bread, I [2]	10	439	27.6	10.7	5.5	24.8	5.36	4.14	4.72	14.22
Peeled wheat bread	5	629	26.1	16.6	19.3	25.7	13.40	18.40	9.98	41.78
Peeled wheat bread, high vitamin yeast	5	609	25.0	17.6	20.9	25.9	13.40	22.9	13.19	49.49
White bread, 5% non-fat milk solids	5	512	24.4	10.9	4.6	17.3	6.02	4.75	8.28	19.05
Lean white bread, II	5	553	22.3	11.6	3.7	24.7	6.49	5.06	5.74	17.29

[1] Shredded Wheat Biscuit of the National Biscuit Company.

[2] Eaten with extra B vitamins.

TABLE 3

Average daily intake in grams and apparent digestibility calculated as per cent of intake.

TEST FOOD	NO. OF SUBJECTS	TEST FOOD	ASH		CARBOHYDRATE		FAT		INDIGESTIBLE RESIDUE	
			Intake	App. dig.	Intake	App. dig.	Intake	App. dig.	Intake	App. dig.
		gm.	gm.	%	gm.	%	gm.	%	gm.	%
Egg	10	230	6.47	63.5	302	99.7	151	97.0	3.47	29.2
Whole wheat cereal biscuit [1]	10	254	8.95	46.4	270	97.6	140	95.7	25.87	48.2
Locally made whole wheat bread, 5% non-fat milk solids	10	281	10.57	70.5	243	98.7	144	97.1	16.41	47.0
Peeled wheat bread [2]	10	282	11.45	73.5	243	99.1	158	97.1	18.48	59.9
Lean white bread, I [2]	10	337	11.31	84.4	270	99.7	171	97.4	9.48	75.4
Peeled wheat bread, high vitamin yeast	5	284	11.48	74.4	242	98.5	145	97.0	18.57	55.0
Peeled wheat bread	5	279	11.30	75.3	231	98.6	147	97.1	18.38	61.7
White bread, 5% non-fat milk solids	5	319	11.31	83.6	269	99.8	152	97.0	9.81	68.1
Lean white bread, II	5	327	10.70	62.1	267	99.8	143	97.2	10.90	76.3

[1] Shredded Wheat Biscuit of the National Biscuit Company.

[2] Eaten with extra B vitamins.

a striking uniformity of mineral intake occurred. In view of this finding it is regrettable that time did not permit complete mineral balances to be made in this experiment.

It is evident that the absorption of fat and carbohydrate is excellent in all instances. It is thus obvious that the extra indigestible residue of the whole wheat or peeled wheat bread as contrasted with the white breads does not interfere with the absorption of these two major foodstuffs. This result is in agreement with those of Adolph and Mao-Yi Wu ('34) as regards the influence of fiber on the digestibility of protein in the absence of a laxative effect.

The percentages recorded in the last column represent the relative absorption or, perhaps better, the disappearance of the indigestible residue. In view of present ignorance concerning the chemistry and digestive fate of the residue components, these values can hardly be interpreted as indicating digestive utilization. The authors are not alone in finding apparently high values for "absorption" of "indigestible residue." For example, Heller and Wall ('40) reported coefficients of utilization of indigestible residue of 16 to 52% when cereal feeds were fed to rats. These values obtained by a modified Williams and Olmsted method may be compared with the range of 29 to 76% found in this experiment. For a more detailed discussion of this question and that of accuracy obtainable the former paper as well as that of Crampton and Maynard ('38) should be consulted.

An additional example of this phenomenon should be cited. Shepherd, Hummel and Macy ('40) have reported the disappearance of these same constituents in connection with their studies on children. The values recorded range from 59 to 91% of cellulose and 37 to 73% of the hemicellulose ingested. It is certain that the final solution of this problem must await more accurate chemical characterization and investigation of the fate of these materials in the intestinal tract.

Special attention is drawn to the parallelism between the nitrogen and the total indigestible residue in the feces, as related to wet weight of feces, illustrated in figure 1. On each

curve the lower points pertain to the values for the white breads, the middle group of points to those for the whole wheat breads, and the extreme upper right-hand group for the whole wheat cereal biscuit. This parallelism is strongly confirmatory of the concept which originated with the Voit school of physiologists that the nitrogen content of the feces

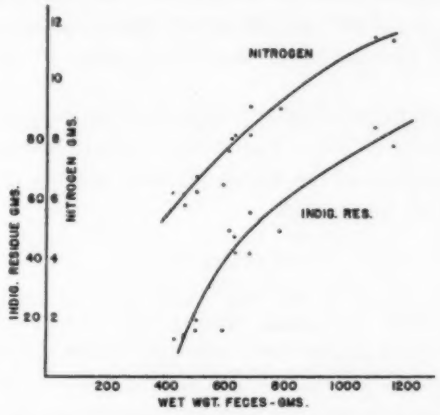

Fig. 1 Parallelism between total indigestible residue and fecal nitrogen in their relation to total moist weight of feces.

is more closely related to the endogenous (alimentary) products than to the nitrogen content of the foods. In this conception indigestible residue is related to these endogenous products as an excitant for the production of increased alimentary secretion. The difficulties attendant to the exact separation of food N from alimentary N in the feces are not wholly overcome by use of the biological value method, as all students of this problem agree.

SUMMARY

The effect of white and whole wheat breads and shredded wheat on the apparent digestibility of fat, carbohydrate and "indigestible residue" has been determined with ten men as subjects.

The apparent digestibility of carbohydrates varied from 97.6 to 99.8% and of fat from 95.7 to 97.4%. No significant difference between the results obtained with white breads and whole wheat bread was found.

It may be concluded that under the conditions of these experiments in which the diet furnished approximately adequate calories for maintenance of weight of each subject, the higher "indigestible residue" of the whole wheat products does not interfere with the digestion and absorption of carbohydrate and fat.

The "indigestible residue" exhibited an apparent digestibility of 29.2 to 76.3%. The white breads gave the higher values, 68 to 76%, and the whole wheat products the somewhat lower values of 55 to 62%.

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DARK ADAPTOMETER AND BLOOD VITAMIN A MEASUREMENTS IN A NORTH CAROLINA NUTRITION SURVEY

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ONE FIGURE

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The purpose of this investigation was to measure the amount of carotene and vitamin A in the blood of subjects whose visual threshold had been determined with the Hecht and Shlaer dark adaptometer. A number of instruments have been designed for the purpose of measuring visual adaptation in dim light following exposure to bright light as a test for the state of vitamin A nutrition in man (Jeans and Zentmire, '34; Edmund and Clemmesen, '36; Feldman, '36; Jeans et al., '37; Hecht and Shlaer, '38; Wald, Jeghers and Arminio, '38; Pett, '39; Haig and Lewis, '39; Steele, '40). The above-mentioned dark adaptometer is one of the most promising of these since it permits determination of the visual threshold throughout the process of dark adaptation, and it meets certain specifications enumerated by the authors as essential for measurement of visual thresholds, that have not been met in earlier instruments.

During the past 15 years much evidence has been obtained to establish a definite relationship between night blindness and vitamin A nutrition. Holm ('25) devised a jumping test for

[1] From a thesis submitted by M. E. Yarbrough in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Duke University.

vision in rats and showed that night blindness is an early symptom of vitamin A deficiency, and Fridericia and Holm ('25) found that the rate of regeneration of visual purple in the retinas of rats is much slower in those deprived of vitamin A than in normal animals. In 1935 Wald demonstrated that vitamin A combines in the retina with a protein to form visual purple, which in the presence of light changes to visual yellow. A part of the visual yellow is resynthesized to visual purple and the other part decomposes into vitamin A and a protein. Some of the vitamin A is destroyed and must be replaced by vitamin A from the blood. In avitaminosis-A this cycle is retarded, the visual threshold is raised and night blindness results. From these findings it seemed logical to use the measurement of visual adaptation as a test for early stages of vitamin A deficiency in man, and the various above-mentioned instruments were devised for measuring visual adaptation and making such tests.

If night blindness is due to a lack of visual purple in the retina which is in turn due to a lack of vitamin A supplied from the blood it seems reasonable to assume that the readings from any instrument that is a satisfactory indicator of vitamin A deficiency should correlate with blood vitamin A. In this investigation the Hecht and Shlaer dark adaptometer was used for measuring the visual threshold levels of subjects whose blood vitamin A and carotene had been determined, and the degree of correlation between the results of the two tests was calculated.

EXPERIMENTAL

The subjects of this study were those included in a Nutrition Survey [2] being made of a typical North Carolina rural mill village. About 60% of the total population, selected without bias as to economic and social grouping, was examined. The ages ranged from 6 to 68 years for the adaptometer measurements, and from 5 months to 68 years for the blood vitamin A

[2] The Cooperative Nutrition Survey in North Carolina is sponsored by the North Carolina State Board of Health in cooperation with the Rockefeller Foundation and Duke University Medical School. The first community survey was in Chatham County.

and carotene determinations. Both tests were made on 154 subjects and the blood tests alone on forty-three additional subjects. These subjects will be referred to as the "nutrition survey group".

Before examining the nutrition survey subjects, determinations were made on a "test group" composed of graduate students and members of the medical school staff. Since this group numbered only sixteen it was not satisfactory for establishing normal values, but the values thus obtained served as a check on the instrument and on the technique of the operator when compared with those reported as normal by Hecht and Mandelbaum ('39) and Isaacs et al. ('40).

Vitamin A and carotene in the blood. Non-fasting blood samples were used throughout this investigation. These samples were taken within 24 hours of the adaptometer test in 34.4% of the cases, within 10 days in 90.4%, and the remainder within the main survey period of 6 weeks. The following evidence indicates that this procedure is justifiable. Kimble ('39) has reported that ingestion of 250,000 I.U. of vitamin A by each of two individuals more than doubled the plasma vitamin A in 2 hours, but that ordinary meals did not cause a measurable increase in either the vitamin A or the carotene within 2 to 6 hours. If no vitamin A concentrate was taken with the meal, then she found that fasting and postprandial samples gave the same levels for vitamin A and carotene. We have extended this observation by administration of 20,000 I.U. of vitamin A together with feeding a light breakfast devoid of vitamin A rich foods to each of sixteen normal adult subjects, followed by measurement of the plasma level at intervals. The highest level of vitamin A in the plasma was generally found at the second or third hour after the meal and the average increase over the fasting level was 30%.

The amount of vitamin A ingested with these experimental meals may be compared with the intake of the nutrition survey subjects. Their average daily intake was 3,500 I.U., and only seven ingested more than 10,000 I.U. daily, the highest intake being 14,500 I.U. This intake was largely in the form

of carotene which is known to be less efficiently absorbed from the intestine than the colorless vitamin A. From these figures we conclude that the ingestion of vitamin A by the nutrition survey subjects at any single meal was not large enough to cause an increase in vitamin A of the plasma greater than the error of the determination. This conclusion is strengthened by the observation that for the twelve subjects whose vitamin A intake was greatest, the mean vitamin A content of the plasma was 52 I.U. per 100 ml., compared with 51 I.U. for the entire group of 154.

The method employed for determining the vitamin A and carotene of the plasma is essentially that of May et al. ('40). The datum L_{440} was used as a measure of the carotene, and L_{620} as a measure of vitamin A. From L_{440} the carotene content of the plasma in micrograms per 100 ml. plasma was obtained on multiplying by the factor 694. From L_{620} allowance for the blue color produced by the carotene and for meniscus error of the solution in the open colorimeter cell was first made by calculating L_{620} (corrected) $= 1.43\, L_{620} - 0.14\, L_{440}$. The determination of the constant 1.43 will be described in a later article; the constant 0.14 was established by Dann and Evelyn ('38). The corrected L_{620} was then multiplied by the factor 600 to convert it to International Units of vitamin A per 100 ml. plasma.

The mean and standard deviation of the determinations for both the test group and the nutrition survey group are given in table 1. Table 2 gives the normal values reported by other investigators who have used a similar method of extraction and a photoelectric colorimeter for color measurements. It is seen that the carotene values for both the test group and the nutrition survey group, and the vitamin A figure of the former, compare favorably with the other recorded figures, but the blood vitamin A value of the nutrition survey group is slightly below these reported for the normals. This finding accords well with the data of the dietary survey of these subjects, which showed that no food particularly rich in vitamin A was

used by them and that the carotene of green vegetables provided the greater part of the vitamin A value of their diets.

Method of dietary survey. This survey was carried out by the staff of the Cooperative Nutrition Survey in North Carolina, and a detailed account will be published elsewhere. Each subject was provided with a form for recording all foods ingested during a period of 1 week, and instructed how to fill it

TABLE 1

	Test Group			*Nutrition Survey Group*		
MEASUREMENT MADE	NO. SUBJECTS	MEAN AND STANDARD ERROR	RANGE	NO. SUBJECTS	MEAN AND STANDARD ERROR	RANGE
Visual threshold (log threshold $\mu\mu$l.)	16	3.012 ±0.037	2.799 to 3.264	154	2.936 ±0.021	2.337 to 3.800
Blood carotene (μg. per 100 ml. plasma)	16	183 ±18.4	96 to 327	197	131 ±4.232	34 to 422
Blood vitamin A (I.U. per 100 ml. plasma)	16	71 ±3.3	44 to 88	197	51 ±1.23	7 to 101

TABLE 2

Carotene and vitamin A values of human blood.

	CAROTENE μG. PER 100 ML. PLASMA	VITAMIN A I.U. PER 100 ML. PLASMA
Kimble ('39)	166 for men range 50 to 300 186 for women range 90 to 340	127 for men range 89 to 198 91 for women range 67 to 169
May et al. ('40)	135 to 525 children 2 to 12 yrs.	49 to 164
Caveness et al. ('41)	Mean 230 stand. dev. 103	Mean 68 stand. dev. 16.5
Sobotka ('41)	100 to 250	60 to 80
Murrill et al. ('41)	Mean 213 stand. dev. 72	Mean 93 stand. dev. 15
Test Group	Mean 183 stand. dev. 74	Mean 71 stand. dev. 13.2
Nutrition Survey Group	Mean 131.2 stand. dev. 59.4	Mean 51 stand. dev. 17.2

out. He or she was seen daily during the recording period and the record checked for accuracy of the entries made. Children's records were kept by their parents. The only foods providing significant amounts of vitamin A were found to be eggs and green vegetables, and for a few individuals, milk was also important. Amounts of these foods ingested were recorded as number of eggs, tablespoonfuls of vegetables (average weight of tablespoonful being taken from a number of weighings made during the survey), and glasses of milk, at 8 ounces to the glass. The vitamin A values of these foodstuffs were taken from the tables of Sherman and Lanford ('40).

Adaptometer readings. According to Wald et al. ('38) and Hecht and Mandelbaum ('40) the most exact index of the level of vitamin A nutrition which can be obtained by dark adaptation measurements is the rod threshold determined after the subject has been in the dark long enough for virtual completion of dark adaptation. We therefore measured the rod threshold for a retinal area 3 degrees in diameter situated 7 degrees nasally for flashes of violet light 0.2 second in duration. This is the procedure of Hecht and Mandelbaum except that we dispensed with the initial light-adaptation. The subject entered the dark room and the first threshold measurement was made after 28 minutes; subsequent measurements were made at 3-minute intervals until the 40-minute reading. The 40-minute reading was used for the correlation studies.

The mean and standard deviation of the visual thresholds are given in table 1. The thresholds of the test group ranged from 2.78 to 3.26 (log micro-microlamberts). These limits are within the narrow range that includes 80% of the normals of Hecht and Mandelbaum ('39, '40), and well within the range of 1.0 log unit of Isaacs et al. ('40). The range of thresholds among the nutrition survey group is wider, from 2.30 to 3.80. With the exception of three observed thresholds at 3.70 to 3.80, all the remainder may be adjudged normal by the criteria of the two groups of authors just mentioned. It is of interest that the remaining thresholds after discarding these three are

distributed in a way which does not depart significantly from the form of the normal law of error, as judged by calculation of the g statistics of Fisher ('38). But if these three thresholds are included then the g statistics indicate a significant departure from the normal distribution toward skewness.

The graphical representation of the frequency distribution of the nutrition survey group is given in figure 1; as is shown there, the total spread of the final rod threshold values is

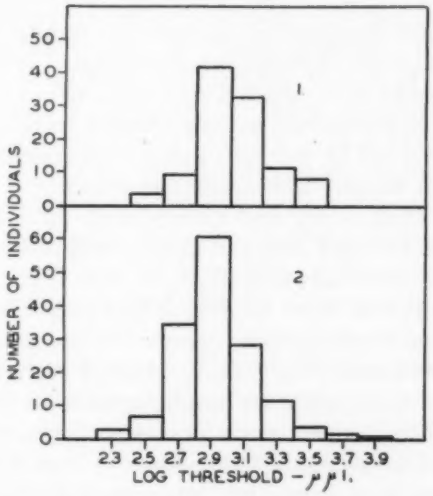

Fig. 1 Distribution of the rod threshold results of (1) 110 normal individuals by Hecht and Mandelbaum and (2) 154 nutrition survey subjects.

1.6 log units. Figure 1 also includes for comparison the distribution of the threshold readings from 110 normal subjects of Hecht and Mandelbaum ('39), with a total range of 1.0 log unit. This same spread of results was found by Isaacs et al. ('40) for sixty-eight normal subjects. Although the readings on the nutrition survey group show a wider range, 96.7% of them are below 3.50 log unit which is the highest value of the Hecht and Mandelbaum subjects. By the standards of these

investigators, it appears that 96.7% of the nutrition survey group subjects are normal.

Correlation coefficients. The correlation coefficients were found to be: (1) for dark adaptation threshold with plasma carotene, $r = +0.158$, and (2) for dark adaptation threshold with plasma vitamin A, $r = +0.131$. For 154 pairs of figures the correlation coefficient must differ from zero by more than 0.157 to reach the 5% level of significance (Snedecor, '40).

DISCUSSION

Assuming that hemeralopia is due to a vitamin A deficiency, it might be expected that the results obtained from a reliable instrument would be correlated with the amount of vitamin A in the blood. Experimental evidence tested on this assumption has been presented by workers using instruments other than the Hecht and Shlaer dark adaptometer.

Steininger et al. ('39) and Caveness et al. ('41) used the biophotometer for dark adaptation determinations. The latter calculated the correlation coefficients and found only slight correlation between blood vitamin A and biophotometer readings; the former reported no correlation from inspection of the results obtained. The "dark adapted visual threshold" readings were made with the biophotometer 15 minutes after exposure to bright light, not at the time when the final rod threshold had been reached but when it was still changing. The conclusion was drawn by Caveness et al. "that the biophotometer is unlikely to prove a suitable instrument for measuring with any precision the degree of vitamin A undernutrition of the human subject".

Pett and LePage ('40) reported a definite correlation of blood vitamin A with the visual test developed by Pett ('39). This is a qualitative test and was made within 1 minute after light adaptation, again at a time when the visual threshold is changing very rapidly. Their method for measuring vitamin A in the blood is also open to objections.

In this investigation no correlation was found between either the Hecht and Shlaer dark adaptometer readings and blood

carotene figures or between the adaptometer readings and blood vitamin A figures. These results make it necessary to conclude that the Hecht and Shlaer dark adaptometer is not reliable for detecting mild avitaminosis A by means of a single test. This conclusion seems to be substantiated by the observation that the nutrition survey group gave blood vitamin A results below normal while 96.7% of the dark adaptometer readings were normal according to the criterion of Hecht and Mandelbaum.

In a recent article appearing since our observations were made, Steven and Wald ('41) have suggested that "a more searching criterion than low or high threshold is needed to estimate vitamin A deficiency". They suggest instead the "vitamin A-labile threshold", which is "one that, whatever its initial value, is lowered at least 0.3 logarithmic unit within 2 weeks of regular vitamin A supplementation". Our experience with hospitalized subjects not reported in this paper fully confirms this view, and we have been using the "vitamin A-labile threshold" as the criterion for deficiency for some time.

Unfortunately the necessity both of treating the subject with vitamin A following the first measurement of visual threshold, and of taking a second measurement, sharply limits the applications of the test. It renders the test impracticable for nutritional surveys of the type provided by our subjects, and it also makes it impossible by this test to select a group of slightly vitamin A-deficient subjects for other observations, since they can only be detected by being cured.

SUMMARY AND CONCLUSIONS

Our results lead to the conclusions:

1. A single measurement of the visual threshold cannot be a reliable indicator of mild avitaminosis A. This is in agreement with the conclusion of Steven and Wald, who suggest the necessity of determining also the stability or lability of the threshold.

2. The level of vitamin A in the blood seems to be the most promising method of detecting mild avitaminosis A by means of a single test, although more data are needed to establish the range of normal and abnormal values.

3. The blood vitamin A and carotene levels are somewhat lower for the rural population tested than have been reported for other groups of normal individuals here and elsewhere.

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MAGNESIUM STUDIES IN CALVES

II. THE EFFECT OF MAGNESIUM SALTS AND VARIOUS NATURAL FEEDS UPON THE MAGNESIUM CONTENT OF THE BLOOD PLASMA[1]

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In a previous paper Duncan, Huffman and Robinson ('35) reported that calves receiving whole milk as the sole ration, with or without the addition of various mineral and vitamin supplements, had low plasma magnesium values. Magnesium intakes as high as 14–16 mg. per kilogram of body weight failed to maintain normal plasma magnesium values on the above type of ration. With one exception (Herman, '36), these results have been substantiated by other workers (Knoop et al., '37, '39; Cave et al., '36; Wise et al., '39; Duckworth, '38–'39). The pathology associated with the low blood plasma magnesium values observed in our calves has been investigated by Moore, Hallman and Sholl ('38). The histologic changes associated with the low plasma magnesium values bear some similarities to those observed in rats (Greenberg, Anderson and Tufts, '36; Schrader, Prickett and Salmon, '37; Cramer, '32).

Many reports have been published on the metabolism of magnesium as affected by a variety of conditions but very few data are available on the relative utilization of the naturally occurring magnesium compounds in feedstuffs as compared to the utilization of the inorganic magnesium salts.

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There has always been some controversy regarding the merits of minerals from organic and inorganic sources in nutrition, but generally speaking, basal rations inadequate in some minerals can usually be supplemented satisfactorily with inorganic sources insofar as the bovine is concerned. This paper reports a study of the comparative value of magnesium salts and various natural feeds upon magnesium metabolism of the growing bovine when fed as a supplement to whole milk ration, in the maintenance of a normal level of magnesium in the blood plasma.

EXPERIMENTAL

The precautions observed in the routine withdrawal of the blood samples, their disposition in the laboratory, and the methods used for the determination of plasma calcium, magnesium and inorganic phosphorus have been recorded (Duncan, Huffman and Robinson, '35). Plasma determinations were made each week and these values were averaged for the 30-day periods which are reported in the tables. The changes in plasma magnesium were often quicker and more pronounced than is indicated by the averaged values.

The basal ration consisted of whole milk, viosterol, iron, copper and manganese. In some instances either corn starch or a cereal breakfast food [2] was fed as an additional source of energy. This ration supplied 10–12 mg. of magnesium per kilogram of body weight. The basal ration was supplemented with either magnesium oxide (light), corn, alfalfa hay, alfalfa ash, corn gluten meal, or cane molasses. When the supplements were added to the basal ration, they were added as such and not on an equal calorie basis. With the exception of four calves, wood shavings were used as bedding. The calves also had free access to the shavings which were their only source of roughage. Four of the animals were placed on wooden slats and given free access to regenerated cellulose as their only source of roughage. Water was offered to the animals twice daily, and they received exercise in a dry lot except during inclement weather.

[2] Rice Krispies.

The viosterol was mixed with the milk at each feeding. No instances of vitamin A or D deficiencies were manifested. The mineral supplement consisted of C.P. ferrous sulfate, C.P. copper sulfate and C.P. manganese sulfate. The minerals were made up in solution and added to the milk at each feeding so that each kilogram of milk contained 16.9 mg. iron, 1.5 mg. copper and 1.3 mg. manganese. The magnesium supplements were also mixed with the milk and the amounts indicated in the tables represent the total magnesium intake. The calves were weighed in the morning before feeding at 10-day intervals.

RESULTS

Although the physiologic range of plasma magnesium may be comparatively wide (2.00 mg. per 100 cc., minimum to 2.80 mg., maximum), the mean magnesium value obtained from 107 normal calves from birth to 18 months of age has been found to be 2.41 mg., with a standard deviation of 0.38 mg. (Duncan, Lightfoot and Huffman, '38). Variations due to the season of the year have also been noted (Duncan, Lightfoot and Huffman, '40). The significance of the magnesium values in the tables are assessed on this basis.

Magnesium oxide as a source of magnesium. Although there were some individual variations in the amount of magnesium required to maintain normal plasma magnesium levels in this group, abnormally large amounts of magnesium were required in all cases. The results shown in table 1 for magnesium oxide are comparable to the results obtained from the use of the carbonate, chloride and phosphate. The efficiency of the citrate, silicate, sulfate and metallic magnesium was markedly reduced. More than thirty calves have been used to study the efficiency of the magnesium salts.

The plasma magnesium values for calf C 284 were subnormal during the basal feeding period. From 70 to 270 days of age the concentration of plasma magnesium returned to normal and remained within normal limits with an average daily magnesium intake of 53 mg. per kilogram of body weight.

TABLE 1

Effects of various magnesium intakes on the plasma magnesium in the calf.

SUPPLEMENT USED	AGE	WEIGHT	MINERAL INTAKE			Mg INTAKE PER KILO BODY WEIGHT	BLOOD PLASMA		
			Ca	P	Mg		Ca	P	Mg
	days	*kg.*	*grams per day*			*mg.*	*mg. per 100 cc.*		
Basal C 284	30	39.8	5.3	3.8	0.49	12.3	12.8	8.17	2.04
	60	51.2	6.3	4.5	0.58	11.3	12.1	7.47	1.74
	69	59.5	6.4	4.6	0.59	9.9	12.0	7.33	1.64
MgO added	90	66.4	7.5	5.5	5.60	84.3	12.3	7.48	2.12
	120	82.9	8.8	6.7	5.84	70.4	11.4	7.35	2.39
	150	104.1	10.7	8.3	6.13	58.9	10.1	6.76	2.48
	180	124.1	11.4	8.8	6.19	49.9	8.7	7.27	2.41
	210	149.8	14.8	10.7	6.26	41.8	10.5	6.87	2.24
	240	180.6	14.7	10.5	6.18	34.2	11.8	8.33	2.30
	270	213.9	15.8	11.3	6.20	29.0	12.1	8.44	2.21
	300	227.3	15.7	11.2	7.88	34.7	11.8	7.86	1.94
	330	230.8	15.7	11.2	9.81	42.5	11.6	7.81	1.96
	360	245.5	16.5	11.8	11.15	45.4	11.4	7.79	1.98
	390	264.7	15.4	11.0	9.46	35.7	11.1	7.83	2.33
	412	268.6	11.7	8.4	7.23	26.9	11.4	9.02	1.77
MgO discontinued	417	262.3	14.2	10.2	1.31	5.0	10.1	8.39	1.75
	450	248.5	6.2	4.4	0.57	2.3	8.7	11.42	1.65
	455 [1]	239.1	6.2	4.4	0.57	2.4	6.5	16.26	1.40
Basal A 37	18	46.8	4.5	3.5	0.43	9.2	12.1	8.33	2.27
Molasses added	30 [2]	51.8	6.2	4.1	1.16	22.4	12.2	6.94	2.01
	60	64.5	7.2	4.9	1.25	19.4	11.6	7.07	1.97
	90	84.1	9.3	6.2	1.75	20.8	12.1	7.10	1.73
	120	100.9	10.0	7.1	2.16	21.4	10.8	7.96	1.72
Molasses discontinued, corn added	150 [3]	128.6	10.5	10.1	1.97	15.3	12.4	7.88	1.75
	180	161.8	10.6	10.2	2.20	13.6	11.9	7.75	2.12
	196	176.4	11.6	11.0	2.29	13.0	11.6	8.17	1.91
	210	187.7	12.3	11.7	2.92	15.6	11.9	8.12	2.11
	240	224.1	12.3	11.7	2.92	13.0	11.5	7.89	1.99
	270 [4]	247.3	12.3	11.7	2.92	11.8	11.6	7.87	2.12
Basal plus corn gluten meal A 35	30 [5]	52.7	4.9	3.6	0.46	8.7	11.3	8.14	2.39
	60	68.2	6.6	4.9	0.63	9.2	11.1	8.28	1.87
	90	90.9	7.4	5.6	0.73	8.0	11.4	7.07	1.86
	112	108.6	8.5	6.6	0.87	8.0	11.6	7.13	1.51
MgO added to above	142 [6]	133.2	9.0	7.0	2.12	15.9	11.5	8.93	1.61
	172	158.6	10.8	8.8	2.34	14.8	11.3	9.13	2.07
	202	185.5	11.8	9.5	2.47	13.3	12.2	8.26	1.93
	232 [7]	206.8	11.1	10.3	3.10	15.0	12.0	7.30	2.10
	249	219.1	11.5	10.3	3.11	14.2	11.9	7.53	1.52
	262 [8]	229.1	14.1	11.7	3.98	17.4	11.9	7.85	2.10
	292	253.2	14.1	11.7	3.98	15.7	11.7	6.99	1.82
	322 [9]	274.1	14.1	11.7	3.98	14.5	11.9	7.01	2.05

[1] Slaughtered.

[2] Increasing amounts from 0.5 to 0.9 pounds per day.

[3] 2 pounds per day to 196 days of age, 3 pounds thereafter.

[4] Changed to another experiment.

[5] Intake of CGM increased progressively from 0.1 to 1.0 pound per day to 112 days of age.

[6] 2 gm. MgO plus 2.0 pounds CGM per day to 232 days of age.

[7] 3 gm. MgO plus 2.5 pounds CGM per day to 262 days of age.

[8] 4 gm. MgO plus 3.0 pounds CGM per day to 322 days of age.

[9] Changed to another experiment.

The values from 270 to 412 days of age dropped below the normal range (less than 2.0 mg. per 100 cc.) when the average daily intake was reduced to 37 mg. per kilogram. The growth curve for C 284 was approximately normal until the magnesium supplement was discontinued at 413 days of age, after which time the body weight and the plasma magnesium values decreased markedly until the calf was slaughtered at 455 days of age.

Attention should be directed to the high plasma inorganic phosphorus values obtained during the latter stages of the experiment. These unusually high values have been observed in nearly all cases where the calves had been maintained on whole milk rations and were associated with pathologic kidneys. The most characteristic feature of the renal inadequacy was the retention of inorganic phosphorus in the blood. The failure of its excretion denoted an advanced and grave condition which usually terminated in death. Some degree of hypocalcemia was evident in many calves which had advanced renal damage but the concentration of plasma magnesium was not influenced to any extent.

Corn as a source of magnesium. Six calves were used to demonstrate the efficiency of corn when fed as a supplement to a whole milk ration in maintaining normal plasma magnesium levels. Data are presented in table 1 for calf A 37. At 19 days of age cane molasses was added to the whole milk ration. The addition of the molasses, which more than doubled the magnesium intake over the basal feeding period, was insufficient to meet the magnesium requirements. At 121 days of age, the molasses was omitted from the ration and 2 pounds of no. 2 yellow corn were substituted for the next 74-day period. This amount of corn was sufficient to increase the plasma magnesium to approximately normal levels. After 196 days of age, 3 pounds of corn per day were fed to maintain the plasma magnesium levels at the lower limits of the normal range until the calf was changed to another experiment at 270 days of age. The data obtained from the other calves in this group showed some individual variations but the results

of the group as a whole indicate that total magnesium intakes from 12 to 15 mg. per kilogram of body weight were sufficient to maintain normal growth and plasma magnesium values.

Corn gluten meal as the source of magnesium. Table 1 shows the effect of supplementing the whole milk ration of A 35 with corn gluten meal. The ingestion of from 0.1 to 1.0 pound per day in addition to the whole milk was ineffective in maintaining the plasma magnesium at normal levels. When small amounts of magnesium oxide were added to the ration to increase the total magnesium intake to 13–18 mg. per kilogram of body weight, the corn gluten meal then supplied the needed requirement to maintain normal plasma magnesium values. These small amounts of magnesium oxide were not effective, however, without the corn gluten meal. The appetite of the calves for corn gluten meal was quite poor and some difficulty was encountered in getting them to consume large amounts.

Alfalfa hay as a source of magnesium. Four calves were fed alfalfa hay as a supplement to the basal ration as the source of magnesium. The experimental data for calf C 401 are presented in table 2. This experiment may be divided conveniently into three periods, from birth to 180 days, from 180 to 330 days, and from 330 to 582 days of age during which the magnesium intakes averaged 30 mg., 16 mg., and 10 mg. per kilogram of body weight respectively. The results obtained with this calf and others in the group show that even the smallest supplement of hay per kilogram provided sufficient magnesium to meet the requirement for the maintenance of normal levels of plasma magnesium.

Alfalfa ash as the source of magnesium. Table 2 shows the results of supplementing the basal ration of calf C 273 with alfalfa ash. The efficiency of a small amount of alfalfa hay in maintaining normal plasma levels suggested the idea of destroying the organic material present in the hay and feeding equivalent amounts of the ash. A large amount of alfalfa was, therefore, burned in the air, and the ash was collected and fed as a supplement to the basal ration. The results indicate that 30–40 mg. of magnesium per kilogram of body weight

TABLE 2

Effects of magnesium in alfalfa hay and alfalfa ash on the plasma magnesium in the calf.

SUPPLEMENT USED	AGE	WEIGHT	MINERAL INTAKE			Mg INTAKE PER KILO BODY WEIGHT	BLOOD PLASMA		
			Ca	P	Mg		Ca	P	Mg
	days	*kg.*	*grams per day*			*mg.*	*mg. per 100 cc.*		
Basal plus alfalfa hay	30[1]	59.0	9.1	4.2	1.36	23.0	11.2	5.04	2.85
	60	70.3	13.3	5.2	2.20	31.3	11.0	6.06	2.29
	90	87.5	14.1	5.8	2.27	25.9	12.5	7.04	2.20
	120	105.2	20.7	8.3	3.58	34.0	12.4	7.73	2.23
	150	118.8	24.4	8.9	4.29	36.1	12.5	8.98	2.67
	180[2]	152.4	25.3	10.2	4.69	30.8	11.9	8.27	2.78
	210	174.6	18.1	9.6	3.19	18.3	11.4	8.84	2.44
	240	198.2	15.0	9.9	2.76	13.9	11.5	8.44	2.12
	270	224.5	19.7	10.5	3.82	17.0	11.4	9.26	2.23
	300	257.6	21.9	10.7	4.31	16.7	11.7	8.50	2.43
	330[3]	279.9	18.6	10.3	3.57	12.8	11.9	7.80	2.00
	360	297.6	18.3	10.3	3.49	11.7	11.6	8.63	2.11
	390	317.5	18.3	10.3	3.49	11.0	11.4	8.83	2.50
	420	343.4	16.2	10.3	3.11	9.1	11.3	8.16	2.41
	450[4]	357.2	15.6	10.3	2.88	8.1	11.4	8.72	2.09
	462	363.2	17.4	11.1	3.29	9.1	11.2	8.36	1.95
	492	389.1	20.3	10.6	4.58	11.8	11.0	7.99	2.61
	522	394.5	20.8	10.5	3.64	9.2	11.4	7.74	2.75
	552	411.8	20.4	10.5	3.49	8.5	11.2	7.71	3.07
	582	419.1	20.9	10.7	4.63	11.0	11.1	8.34	2.56
Basal C 273	30	46.1	5.6	4.0	0.52	11.3	11.9	7.21	2.14
	60	59.8	7.0	5.0	0.65	10.9	12.2	7.95	2.00
	90	78.2	8.6	6.3	0.87	11.1	12.3	8.00	1.81
	120	98.5	10.1	7.5	1.04	10.6	12.2	8.05	1.81
	134	114.5	10.6	7.8	1.04	9.1	11.2	8.65	1.61
Alfalfa ash added	150	112.5	25.0	9.4	4.35	38.1	11.2	8.52	2.16
	180	148.2	25.1	9.6	4.43	29.9	11.4	8.41	2.31
	210	165.0	25.2	10.0	4.62	28.0	11.1	8.48	1.85
	240	187.3	39.3	12.2	8.06	43.0	11.2	8.25	1.78
	270	210.6	41.2	13.3	8.31	39.5	11.2	8.39	1.96
	300	245.2	43.7	13.9	7.93	32.3	11.3	8.18	1.91
	330	267.4	43.6	13.8	7.89	29.5	11.1	8.72	1.61
	360	288.8	44.4	14.9	8.02	27.8	11.2	8.36	1.46
Ash discontinued. MgO added	366	295.9	16.8	12.0	1.55	5.2	10.2	6.87	1.38
	390	305.8	16.8	12.0	11.20	36.6	10.7	8.21	1.71
	420	318.6	17.2	12.3	11.24	35.3	10.6	8.90	2.25
	450	329.1	17.9	12.8	11.30	34.3	10.4	9.45	2.29
	480	358.6	18.0	12.9	11.31	31.5	10.5	8.57	2.19
	490[5]	368.2	18.9	13.6	11.39	31.0	10.6	8.34	2.08

[1] Intake of alfalfa increased progressively from 0.4 to 2.0 pounds per day to 180 days of age.

[2] Variable amounts of alfalfa from 0.6 to 1.5 pounds per day to 330 days of age.

[3] 1.0 pound alfalfa per day to 450 days of age.

[4] 1.5 pounds alfalfa per day to 582 days of age. Changed to another experiment.

[5] Changed to another experiment.

in the form of alfalfa ash were not as effective as the same amount of magnesium in alfalfa hay. Intakes of 28–38 mg. per kilogram were sufficient to maintain normal plasma magnesium values for a short time but similar intakes failed to maintain the normal level for extended periods. The feeding of an equivalent amount of magnesium in the form of the oxide restored the plasma magnesium to normal levels. In three other calves, the ingestion of more than 38 mg. of magnesium per kilogram per day in the form of alfalfa ash was insufficient to maintain the level of plasma magnesium within the normal range.

DISCUSSION

The results of this investigation indicate that the utilization of magnesium by growing calves is more efficient when magnesium is furnished by natural feeds than by magnesium salts. The low plasma magnesium values and the symptoms observed following the long-continued feeding of a basal ration of whole milk, viosterol, iron, copper and manganese were prevented by supplementing the basal ration with either magnesium carbonate, oxide, phosphate or the chloride. An important observation in this work, however, was the large amount of dietary magnesium that was required to maintain normal plasma magnesium levels when magnesium salts were used as supplements. The feeding of 20–30 mg. of additional magnesium, bringing the total intake up to 30–40 mg. per kilogram, was required. The results of these experiments confirm those of Blakemore and Stewart ('34–'35), Allcroft and Green ('34), and Knoop et al. ('39) in showing that the feeding of magnesium salts to calves does not raise the plasma magnesium level above normal, contrary to the observations reported by Cunningham ('33, '34) on sheep and cows, and by Johnson, Palmer and Nelson ('40) on sheep.

When yellow corn was fed as a supplement to the basal ration, the total intake of 12 to 15 mg. of magnesium per kilogram of body weight was sufficient to maintain normal plasma magnesium values. Even better results were obtained with

alfalfa hay but more than 30 mg. of additional magnesium per kilogram failed to maintain the normal level when alfalfa ash was fed as a supplement to the whole milk ration. The results obtained from feeding corn gluten meal alone were inconclusive but when additional amounts of magnesium oxide were added to raise the total magnesium intake above 13 mg. per kilogram, normal plasma magnesium values were obtained and the calves grew at a normal rate. These results suggest that the factor, or factors, responsible for the decrease in the amount of magnesium required to maintain normal plasma magnesium values is organic in nature and is present in natural feeds such as alfalfa, corn, and corn gluten meal.

The results obtained from feeding calves a whole milk ration supplemented with cane molasses are contrary to those reported by Cunningham ('34) and Eveleth and Eveleth ('35) who stated that a drench of crude molasses or glucose elevated the serum magnesium in sheep and pigs. In all cases, the addition of molasses to the ration of young calves failed to maintain normal plasma magnesium values. The hypomagnesemia resulting from the whole milk ration appeared to be intensified upon the addition of the molasses. Since milk is high in lactose and molasses is high in sucrose, it does not appear logical that the beneficial results obtained from the feeding of alfalfa, corn or corn gluten meal can be explained on the basis of the sugar content of either supplement.

The failure of 10–12 mg. of magnesium per kilogram of body weight to maintain normal plasma magnesium values in calves fed whole milk as the sole source of magnesium is of additional interest in view of the observation of Greenberg and Tufts ('38) that the magnesium requirement of the rat is satisfied by an intake of 5 mg. per 100 gm. of food. These investigators also reported that the minimal requirement is raised to some extent by a high calcium or low vitamin G intake. Milk is high in calcium which would appear to explain the results obtained with corn but would not account for the results obtained with alfalfa hay which contains from 1.5–2.0% calcium. Milk is also high in vitamin G (riboflavin) so that a deficiency

of this factor is probably not responsible for the higher magnesium requirement of the calf when milk is used as the sole ration. Other members of the B-complex are not entirely ruled out, however, although most of these factors are considered to be synthesized in the rumen of the ruminant (McElroy and Goss, '39). Day and Orent-Keiles ('39) reported that rats fed whole milk ad libitum, supplemented with thiamine, iron, copper and manganese failed to develop the symptoms of magnesium deficiency. The results with rats, however, are not comparable to the results obtained with calves because rats usually consume from two to three times as much milk per kilogram of body weight as do calves. Our results suggest that milk may be deficient in some factor necessary for normal magnesium metabolism in the young bovine.

The data presented in the tables indicate that the average daily intake of calcium and phosphorus and the calcium: phosphorus ratio of the whole milk ration alone or with the various supplements provided an adequate amount of these elements for the normal growth of young bovine. The vitamin D content of the milk and viosterol, plus exposure to direct sunlight, was sufficient to permit normal calcium and phosphorus metabolism and to maintain normal plasma calcium and inorganic phosphorus levels until the kidneys became pathologic. The calcium: magnesium ingestion ratios indicate that they are not the influencing factors in the maintenance of normal plasma values. The magnesium supplement in the form of alfalfa hay, corn or corn gluten meal had calcium: magnesium ingestion ratios varying from 3.5 to 6.5: 1 which were satisfactory in maintaining normal plasma magnesium levels. The plasma calcium level was unaltered on both the high and low magnesium intakes. Hypomagnesemia in calves, therefore, cannot always be correlated with a low dietary intake of magnesium since it is unrelated, in a direct manner, with the magnesium content of the ration.

SUMMARY

A basal ration consisting of whole milk supplemented with iron, copper and manganese, with or without the addition of

starch or a carbohydrate rich cereal product always produced hypomagnesemia in calves.

The total intake of 12–15 mg. of magnesium per kilogram of body weight was sufficient to maintain normal plasma magnesium levels when small amounts of either corn, alfalfa hay or corn gluten meal were included in the basal ration.

A total intake of 30 to 40 mg. of magnesium per kilogram was required to maintain normal plasma values, when magnesium salts were added to the basal ration. The same amounts of magnesium in the form of alfalfa ash were less effective than the magnesium salts.

These data suggest that the utilization of magnesium by growing calves is more efficient when magnesium is furnished by natural feeds than when supplied by magnesium salts.

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THE RELATIVE ASSIMILATION OF FLUORINE FROM FLUORINE-BEARING MINERALS AND FOOD (TEA), AND FROM WATER AND FOOD [1]

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The extent to which fluorine in food materials is absorbed from the alimentary tract of animals and retained in the tissues undoubtedly determines the extent of its physiological effect on the body. The assimilation of fluorine may be dependent upon the chemical combinations in which the fluorine occurs (Lawrenz, Mitchell and Ruth, '39 b, '40 a). Changes occur- of nutrients with which it is associated in the diet (Lawrenz and Mitchell, '41), and the manner in which it is administered (Lawrenz, Mitchell and Ruth, '39 b, '40 a). Changes occurring in the animal body itself (Lawrenz, Mitchell and Ruth, '40 b) may also modify the assimilation of food fluorine. As the references cited indicate, this laboratory has made contributions to the various phases into which the problems relating to the assimilation of fluorine may be subdivided. The investigation to be reported in this paper represents an extension of this program, and is specifically concerned with a comparison at low levels of intake of the assimilation of fluorine in sodium fluoride, in calcium fluoride, in raw rock phosphate and in a high-fluorine food (green tea), and also of the fluorine in sodium fluoride administered in water and

[1] The authors gratefully acknowledge the assistance rendered to this investigation by the donation of funds by the Graduate School of the University of Illinois.

in food. The latter problem of the relative toxicity of fluorine in water and in food has previously been studied using cryolite as the source of fluorine (Lawrenz, Mitchell and Ruth, '39 b), but the practical importance of the problem, especially with reference to the significance of evidence of the toxicity of fluorine-bearing waters to an appraisal of safe concentrations of fluorine in foods, is such as to warrant further study.

The relative toxicities of different fluorine compounds are not constant, but vary with the amounts fed, such that the lower the concentration of fluorine in the diet the less variation there is in toxicity (Smith and Leverton, '34; Evans and Phillips, '39). Hence, at levels of fluorine of significance in human nutrition, marked differences in the toxicities of different fluorine compounds are not to be expected, and have not been observed in the comparison of cryolite (synthetic) with calcium fluoride (Lawrenz, Mitchell and Ruth, '39 a). However, not many comparisons of different fluorine compounds have been made at the low levels of intake of significance to the problem of the prevailing fluorine hazard in human nutrition.

This fluorine hazard is a resultant of the natural contamination of water supplies with fluorine, of the industrial contamination of certain foods with fluorine-containing insecticides, and of the natural occurrence of fluorine in foods. McClure ('39) has recently published a compilation of reported fluorine analyses on a considerable number of human foods, from which it may be inferred that, although this element is widely distributed among foods, it occurs in significant amounts only in teas and sea foods, with the exception of some analytical results on foods grown in certain fluorite areas, probably involving dust contamination.

The assimilability or toxicity of the fluorine in these naturally high-fluorine foods appears worthy of study. Reid ('36) has shown that the fluorine in Chinese teas is largely extracted in a 2% infusion of the leaves, and that the fluorine thus extracted when fed at comparatively high levels to young rats is capable of causing the white striations in the incisor teeth

characteristic of fluorosis. Lee and Nilson ('39) have reported experiments on the assimilation of fluorine by rats from canned salmon and mackerel, purporting to show that inorganic fluorine is three times as effective in producing tooth striations as the naturally-occurring fluorine in these sea foods, but the quantitative relations in the assimilation of fluorine from the various experimental diets used in this study can hardly be assessed in view of the wide differences in fluorine concentrations. Since in fish and mammals most of the fluorine is present in the skeleton, and since Ellis and Maynard ('36) have detected no difference in the toxicity of fluorine in sodium fluoride and in bone meal at low levels of intake, one would not expect the fluorine in fish to be appreciably less toxic than that in inorganic fluorides at nutritionally significant levels of intake. The marked difference that Coulson, Remington and Lynch ('35) noted in the assimilability of the arsenic in sea food (shrimp) and in inorganic arsenic would not be expected to hold for fluorine, but evidently the problem of fluorine assimilation from natural foods needs much further study by accurately quantitative methods.

PLAN OF EXPERIMENTS

Experiment 1. The first experiment involved a three-way comparison of calcium fluoride and sodium fluoride administered in water solution, and sodium fluoride administered in food. Thirty young rats, separated into trios on the basis of litter membership, sex and similarity of body weight, were used in this comparison. All rats received a basal diet of corn 20, starch 15.5, sucrose 5, dried yeast 8, dried skim milk 27, casein (Argentine) 8, butter 6, lard 6, cod liver oil 2, wheat germ oil 0.5 and salts [2] 2%. In each trio the rats were fed equal amounts of this food throughout the experiment. The first rat in each trio was given drinking water containing about 7.8 p.p.m. of fluorine in the form of calcium fluoride. The second rat in each trio received the same amount of drinking water containing an equal concentration of fluorine as sodium

[2] Wesson (Science, vol. 75, p. 339, 1932) salts with NaF omitted.

fluoride. The fluorine-bearing water was so apportioned to the rats as to provide approximately 9 p.p.m. of fluorine on the basis of the food consumed. The third rat in each trio received daily the same amount of the same sodium fluoride solution as was given to the second rat, but in this case it was added to the food.

In order to separate as widely as possible the time of ingestion of fluorine in water and fluorine in food for the purpose of securing the maximum effect of the difference in method of administration, all rats were given access to food only from 4 to 5:30 P.M. No water was allowed at this time except the small amounts required to moisten the food to prevent scattering (roughly equal to the weights of food). Access to water was permitted only between 9 and 11 A.M.

The rats were weighed weekly and, after 6 weeks of feeding, their teeth were examined weekly for the appearance of striations, using a jeweler's lens with a magnification of 4 ×. At the end of the fourteenth week of feeding the rats were all killed with ether, and the carcasses autoclaved and skeletonized in the customary manner. The bones, teeth and soft tissues were analyzed for fluorine by methods previously described (Lawrenz, Mitchell and Ruth, '39 a). Check rats selected from the litters providing experimental rats were sacrificed and analyzed for fluorine at the beginning of the experiment. The average content of fluorine on the live weight was 6.54 p.p.m.

Experiment 2. In the second experiment the assimilability by growing rats of the fluorine in green tea and in Tennessee raw rock phosphate was compared with that of the fluorine in sodium fluoride, all fluorine-bearing supplements being incorporated in the basal diet.

The tea was selected from three varieties, bought on the local market, for its high content of fluorine. Among the samples analyzed were a black tea,[3] containing 65 p.p.m. of fluorine, an orange pekoe,[4] found to contain 80 p.p.m. of fluor-

[3] Lipton.
[4] Tenderleaf.

ine, and a green tea [5] with 130 p.p.m. of fluorine. The latter variety was therefore chosen and a subsequent sample that was used in the experimental diets contained even more fluorine than the first, i.e., 178 p.p.m. The rock phosphate tested contained 3.90% of fluorine and 34.96% of calcium.

Twelve trios of litter mate rats were used in this three-way comparison, and check rats from each litter, totalling twelve, were analyzed and found to contain an average of 5.03 p.p.m. of fluorine. The first rat in each trio received a ration composed as follows: casein (Argentine) 20, salts [6] 4, cod liver oil 1.5, wheat germ oil 0.5, corn oil 8, dried yeast 8, corn 38, sugar 15, starch 4.5 and celluflour 0.5%, with additions of $CaHPO_4$ and NaF in the proportions of 644.3 and 21.7 p.p.m., respectively.

The second rat in each trio received the same diet modified by the substitution of 5% of ground tea leaves for the starch and celluflour and the omission of the $CaHPO_4$ and NaF.

The third rat in each trio received the same diet given to the first rat with the NaF replaced by 228.8 p.p.m. of rock phosphate and the proportion of $CaHPO_4$ reduced to 375 p.p.m.

The three experimental diets were designed to contain equal percentages of calcium and fluorine. The concentration of the latter element averaged 11.8 p.p.m. for each diet.

The rats in the second experiment were controlled and measured as were the rats in the first experiment, and, after each trio had consumed 750 gm. of food per rat, they were sacrificed, dissected and analyzed by the same procedures.

EXPERIMENTAL RESULTS

Experiment 1. The rats in this experiment grew as well as the rigorous conditions imposed would permit, gaining an average of about 1.5 gm. daily. The various diets promoted growth about equally well and no untoward symptoms were noted except the occurrence of slight amounts of blood in the

[5] Tenderleaf.

[6] See footnote 2, page 623.

urine of rat 276, receiving CaF_2 in the drinking water, and rat 295, receiving NaF in the drinking water, on the fifth and fourth days of the test, respectively. A similar hematuria was observed in the feeding of cryolite to rats in the drinking water (Lawrenz, Mitchell and Ruth, '39 b).

Striations of the teeth of rats receiving their fluorine supplements in the drinking water were generally very well developed by the beginning of the eighth week, there being no distinction between the rats getting NaF and those getting CaF_2. The trio-mates receiving NaF in the food showed only incipient dental striations at this time.

The average growth data and the average results of the carcass analyses are assembled in table 1. There were no significant distinctions in any item listed between rats 1 and 2 of the ten trios, receiving NaF and CaF_2 in the drinking water. Evidently fluorine administered in these two forms at low levels of consumption is equally assimilable and presumably equally toxic. However, between the first two rats and the third rat in the various trios, clear distinctions are apparent, indicating a smaller toxicity of fluorine when consumed in food than when consumed in water. The most significant comparisons are those between rats receiving NaF in their drinking water and those receiving the same compound in the same amounts in their food. The statistical analyses of such comparisons are recorded in table 2. With reference to the fluorine content of bones, teeth and soft tissues and to the total fluorine retention, the rats receiving NaF in their water definitely surpassed those receiving NaF in their food. Stated in another way, the assimilation of fluorine was depressed by admixture with food 19.3% in the bone, 14.0% in the teeth, 29.0% in the soft tissues, and 21.5% in the entire carcass. The latter figure compares well with that obtained previously in a similar experiment comparing the assimilation of fluorine consumed as cryolite in water and in food (Lawrenz, Mitchell and Ruth, '39 b). In this case an average depression of 20.4% was observed due to admixture with food. The failure of McClure ('39) to detect any such difference may probably be

TABLE 1

The growth data and the fluorine metabolism data secured in the two experiments. Averages for ten trios in experiment 1 and for twelve trios in experiment 2.

	EXPERIMENT 1. AVERAGES FOR RATS RECEIVING			EXPERIMENT 2. AVERAGES FOR RATS RECEIVING		
	Calcium fluoride in water	Sodium fluoride in water	Sodium fluoride in food	Sodium fluoride	Green tea	Rock phosphate
Initial body weight, gm.	48	48	48	39	39	40
Total gain in weight, gm.	149	151	150	176	166	177
Final body length, mm.	210	213	206	214	214	216
Total food consumed, gm.	648	648	648	750	750	750
Number of food refusals	15	13	11	12	5	12
Total intake of fluorine, mg.	6.56	6.56	6.56	8.80	8.80	8.80
Feeding period, days	98	98	98	86	86	86
Final empty body weight, gm.	197	199	198	215	205	217
Weight of dry, fat-free bones, gm.	10.136	10.289	10.170	10.628	10.516	10.665
Weight of dry teeth, gm.	0.426	0.428	0.432	0.398	0.405	0.396
Weight of soft tissues, gm.	179	182	179	194	184	196
Fluorine in bones, p.p.m.	298	290	238	284	272	258
Fluorine in bones, mg.	2.986	2.951	2.380	3.010	2.847	2.743
Fluorine in teeth, p.p.m.	198	196	167	230	209	199
Fluorine in teeth, mg.	0.084	0.084	0.072	0.091	0.085	0.079
Fluorine in soft tissues, p.p.m.	0.42	0.44	0.32	0.26	0.26	0.26
Fluorine in soft tissues, mg.	0.076	0.081	0.057	0.050	0.048	0.050
Total fluorine in carcass, mg.	3.146	3.116	2.509	3.151	2.980	2.872
Initial fluorine content, mg.	0.312	0.314	0.311	0.188	0.187	0.190
Total fluorine retained, mg.	2.834	2.802	2.198	2.963	2.793	2.682
Total fluorine retained, per cent	43.2	42.9	33.6	33.7	31.7	30.5

TABLE 2

Statistical analysis of fluorine metabolism data.

FLUORINE	EXPERIMENT 1. SODIUM FLUORIDE IN WATER VERSUS SODIUM FLUORIDE IN FOOD				EXPERIMENT 2. SODIUM FLUORIDE VERSUS GREEN TEA				EXPERIMENT 2. GREEN TEA VERSUS ROCK PHOSPHATE			
	Mean difference [1]	Standard deviation of differences	t value	P [2]	Mean difference [3]	Standard deviation of differences	t value	P [2]	Mean difference [4]	Standard deviation of differences	t value	P [2]
In bones, p.p.m.	52.6	31.6	4.99	0.0004	12.2	18.9	2.16	0.027	13.0	13.2	3.25	0.0039
In bones, mg.	0.570	0.209	8.20	0.00004	0.163	0.256	2.11	0.029	0.104	0.245	1.41	0.094
In teeth, p.p.m.	28.7	15.3	5.63	0.0002	20.2	25.0	2.69	0.010	10.7	22.7	1.56	0.073
In teeth, mg.	0.0117	0.0071	4.97	0.0004	0.0066	0.0085	2.57	0.013	0.0058	0.0104	1.85	0.045
In soft tissues, p.p.m.	0.128	0.056	6.85	0.00004		Insignificant				Insignificant		
In soft tissues, mg.	0.0235	0.0106	6.65	0.00004		Insignificant				Insignificant		
Total retained, mg.	0.603	0.218	8.31	0.00005	0.170	0.257	2.19	0.025	0.111	0.257	1.43	0.090

[1] The positive sign indicates that the mean difference favors the sodium fluoride administered in water.

[2] The probability according to Student ('25) that a mean difference as large or larger than that observed, and in the same direction, would result from the fortuitous combination of the many uncontrolled factors undoubtedly operating.

[3] A positive sign indicates that the mean difference is in favor of the sodium fluoride.

[4] A positive sign indicates that the mean difference is in favor of the green tea.

ascribed to the fact that in his experiments no attempt was made to separate in time the consumption of water and of food by the experimental rats, thus permitting the obscuration of the very effect it was desired to measure.

Experiment 2. The rats in this experiment grew somewhat better than those in experiment 1, making gains averaging about 2.0 gm. daily, except for the rats on the green tea ration, which consistently grew at a somewhat slower rate than those on the other two rations. Striations appeared on the incisor teeth of the rats generally at the beginning of the seventh week of feeding, and generally were more completely developed in the rat receiving the diet containing sodium fluoride. With two of the rats, one receiving sodium fluoride and one receiving rock phosphate, this depigmentation of the enamel of the teeth proceeded to completion.

The average results of the experiment are summarized in table 1, and the statistical analyses of differences observed between the sodium fluoride diet and the tea diet, and between the latter and the rock phosphate diet are presented in table 2. On the average, the assimilation of fluorine was most complete from the sodium fluoride diet, least complete from the rock phosphate diet, and intermediate from the green tea diet. The differences between fluorine accumulations in bones and teeth promoted by the sodium fluoride diet and the tea diet were statistically significant, taking a value of P of 0.03 or less as being negligible. The total retention of fluorine also was significantly different in this comparison, being 5.4% less on the tea diet. The soft tissues secured on the two diets revealed no difference in fluorine content that could be attributed to a difference in the source of dietary fluorine. With this one exception, the fluorine of sodium fluoride was also distinctly and significantly more assimilable than the fluorine of rock phosphate, the average difference in total fluorine retention being 9.0% in this case.

A comparison of the results secured on the tea diet with those secured on the rock phosphate diet, reveals a greater

average assimilation of the fluorine of tea in the bones, teeth and total carcass, but the differences are borderline in significance.

SUMMARY AND CONCLUSIONS

Comparisons have been made by controlled feeding experiments on growing rats, involving twenty-two trio groups, of the retention of dietary fluorine from supplements of sodium fluoride, calcium fluoride, green tea and raw rock phosphate, and from sodium fluoride administered in water and in food. In the latter comparison, the consumptions of water and of food were separated in time as much as possible in order to measure the unobscured effect. In the comparison of sodium fluoride and calcium fluoride, the salts were dissolved in water. In all experiments the fluorine was administered at levels sufficiently low (9 to 12 p.p.m.) to be of significance with reference to the fluorine hazard in human nutrition. The data secured support the following conclusions:

1. At these low levels of intake, the fluorine in sodium fluoride is no more assimilable by the animal body, and presumably no more toxic, than the fluorine in calcium fluoride. It is, however, definitely more assimilable (5%) than the fluorine in green tea, which in turn is probably somewhat more assimilable than the fluorine in raw rock phosphate.

2. The fluorine of sodium fluoride, administered in the drinking water at low levels, is 21% more completely assimilated by the animal body than the fluorine of the same compound consumed in the same amounts in the food. In a similar experiment previously carried out with cryolite as the source of fluorine, the depression in assimilation brought about by admixture with food was 20%.

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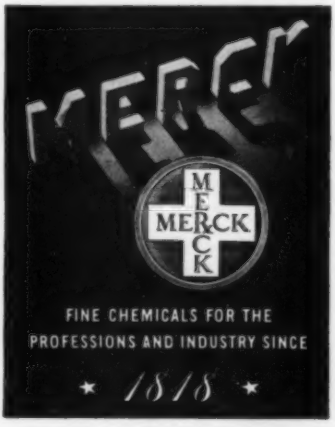

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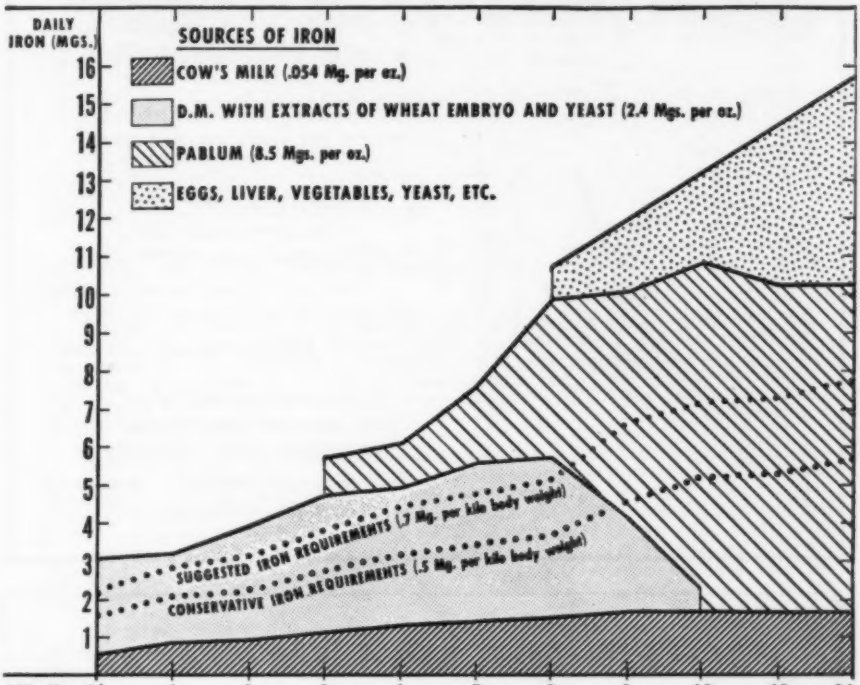

AGE, Mos.	¼	1	2	3	4	5	6	9	12	18	24
WEIGHT, Lbs.	7	9	10	12	14	15	16	20	22	23	25
MILK, Oz.	16	16	18	21	25	26	28	32	32	32	32
D.M. WITH EXTRACTS OF WHEAT EMBRYO AND YEAST, Oz.	1	1	1¼	1½	1½	1¾	1¾	1	¼	0	0
PABLUM, Oz.	0	0	0	⅛	¼	¼	½	¾	1	1	1

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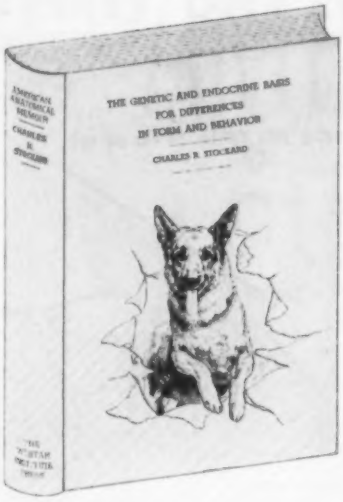

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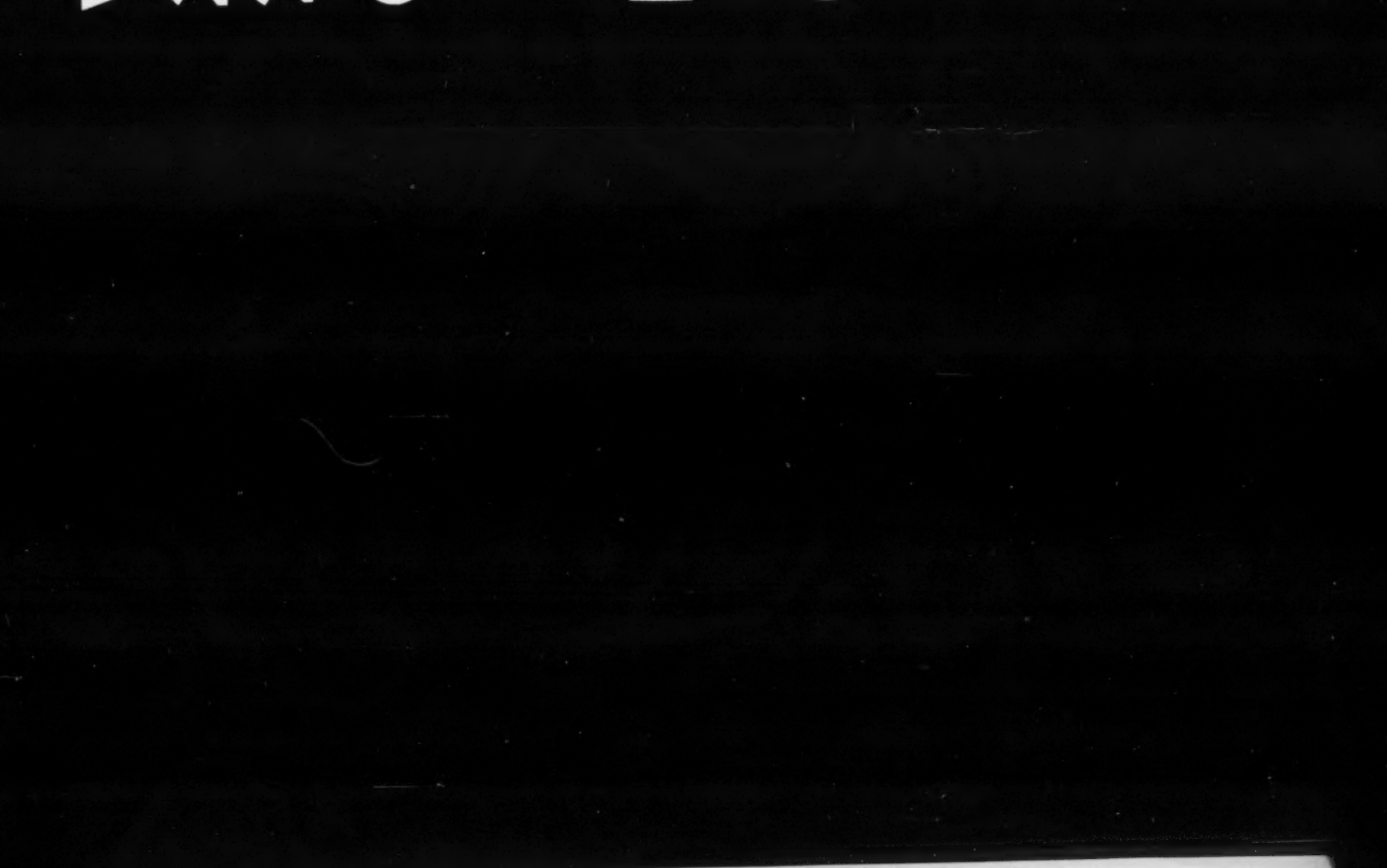

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